CHAPTER-5

DISCUSSION

CHAPTER-5

DISCUSSION

Plants are an excellent source of medicine and have long been used to treat a wide range of illnesses. However, accurate identification of the correct plants is necessary for the effectiveness of medications derived from plants. While traditional taxonomic approaches are still very helpful in identifying medicinal plants and their fruits or leaves for treating different ailments, the herbal sector faces challenges with accurate identification and adulteration of medicinal herbs. The main causes of this are the existence of closely related species and incorrect taxonomic classification. In order to prevent the use of plants that are poisonous, physical characteristics alone are not a sufficient method of identification.

MOLECULAR TAXONOMY STUDY

DNA bar-coding is regarded as a genetic and bioinformatics technique for molecular taxonomy-level classification and identification of plant species. Utilizing a brief genomic DNA fragment, the DNA barcoding technique has proven to be an effective tool for species identification and has been widely applied in molecular plant taxonomy to authenticate species of medicinal plants. By comparing their sequences to reference libraries of constructed barcode sequences, it aids in the authentication of species. DNA barcoding in plants commonly uses nuclear DNA (ITS and ITS2 areas) and plastid DNA (rbcL, matK, trnL and trnH-psbA regions) (Kress *et al.,* 2014; Taberlet *et al.,* 2007). Enough sequence variation exists between closely related species for DNA barcoding to accurately identify them. Nonetheless, universality, sequence quality and coverage are also important factors in identifying the optimal plant DNA barcode locus (Hollingsworth *et al.,* 2009). A functioning DNA barcode requires the primer to be universal (Kress *et al.,* 2005). Three barcode loci-rbcL, ITS1 and ITS2 performed the best in this study's PCR amplification and sequencing tests. Three medicinal plants were selected for their therapeutic efficacy and DNA samples were taken from them to serve as templates. Due to their capacity for species differentiation, the Consortium for the Barcode of Life (CBOL) recommends matK and rbcL regions as a standard two-locus barcode for worldwide plant databases (Fazekas *et al.,* 2012). However, matK is one of the plastid regions that is changing the fastest and there are still many aspects that need to be improved, like primer universality and PCR amplification efficiency (Chase *et al.,* 2007; Hollingsworth *et al.,* 2011). For the chosen plants, other barcode combinations, such matK, were also tried; however, those primers were not recommended for barcoding because of their poor sequencing quality and low discrimination capacity (Thomas, 2009). According to Starr *et al.* (2009), there could be a number of reasons for the low success rate of matK including numerous copies, poor quality sequence data and difficulties with secondary structure development. In the majority of the plant, the rbcL barcode region is simple to amplify and has a high sequencing success rate. Due to its significant sequence divergence, ITS was first suggested as a universal DNA barcode for plants (Kress *et al.,* 2005). It has also been effectively employed as a genetic identifier for molecular authentication and identification of a number of therapeutic plants. Identification of closely related species of herbal plants depends on the higher amount of sequence variation. Numerous metabarcoding investigations have been published that use ITS1 or ITS2 to identify the species of herbal plants (Cheng *et al.,* 2014; Coghlan *et al.,* 2012; De Boer *et al.,* 2017; Raclariu *et al.*, 2017). Potential nuclear DNA barcode Internal Transcribed Spacer (ITS2) is simple to amplify and sequence (Zhao *et al.*, 2018). High discriminating capacity between species of herbal plants and their close relatives has been demonstrated (Chen *et al.,* 2010; Yao *et al.,* 2010). Because of its great variety and capacity to identify species, researchers have suggested using it as a standard DNA barcode identifier for the authentication of herbal plants (Zhang *et al.,* 2018). According to Chen *et al.* (2010), one of the best universal primer combinations for plant identification is rbcL and ITS2. Additionally, ITS2 and rbcL were suggested as significant DNA barcodes for plants because of their strong species identification and discriminating abilities (Kress *et al.,* 2005; Li *et al.,* 2011) and in this work, they did offer ideal species identification. ITS2 and rbcL have previously been proposed as appropriate markers for eukaryotic species classification and phylogenetic reconstruction (Schultz *et al.*, 2005; Coleman, 2007; Miao *et al.*, 2008).

NUTRITIONAL ANALYSIS

Plants have long been valued by humans for their medicinal and nutritive benefits. In food processing, proximate analyses of food and biological materials are critical for evaluating the quality and nutritional composition of food samples. Except for moisture content, all nutritional assessment measurements were performed on a dry basis. Moisture, crude protein, crude fat, ash, crude fibre, total carbohydrate and total energy value are all included in the proximate analysis. In terms of moisture, water determines the energy value (Rahimi and Rabani, 2010).

The moisture content of a species is affected by humidity, temperature, and harvest time. Ogie *et al.* (2001) discovered that increased moisture content enhances microorganism growth and enzyme activity. Results show that the moisture content was moderate and nearly identical in all the studied plant species (the values ranged from 44.97g/100g to 59.4g/100g). Thus, the presence of moderate moisture indicates its reasonable shelf life. The moisture content detected by Banik *et al.* (2018) for *Cinnamomum tamala* is comparable to our findings. The moisture content detected by Payum (2020) is high as compared to our study. Das *et al.* (2023) also reported the high moisture content in some indigenous ethnomedicinal plants. The moisture content of our sample leaves may indicate high storage strength, low susceptibility to microbial infection and a probable reduction in enzyme activities that may hydrolyze bioactive constituents in the pulverized sample (Akpabio and Ikpe, 2013).

Proteins are required for the creation of biological tissues as well as the regulation of substances such as hormones and enzymes (Akindahunsi and Salawu, 2005). Among the plants analyzed, the highest crude protein was found in *Z. oxyphyllum* (24.30g/100g) followed by 15.75 g/100g and 10.75g/100g in *R. serrata* and *B. lanceolaria* respectively. The obtained protein content in the present study is comparable to the finding of Otunola *et al.* (2020). The protein content found in our samples is more as reported by Ogunka-Nnoka, *et al.* (2020) for

Centella asiatica leaves and for wild edible plants as reported by Tharmabalan, (2021). The protein content values recorded for *Z. oxyphyllum* were found to be high to that reported by Belewu *et al.* (2009) for *Ocinum gratissimum* (20.78%). Whereas the protein content for *Z. oxyphyllum* and *R. serrata* were also higher than the value reported by (Olumide *et al.,* 2019) for *Ocinum gratissimum* and *Vernonia amygdalina*. The high protein content values recorded in this study suggest that the plant leaves can be ranked as a potential source of plant protein and therefore be used as a protein supplement in the diet. Protein's importance in various physiological activities (such as hormone production, immune function, and body development, among others) cannot be overstated, as shortages is one of the key reasons contributing to nutritional disease (Achi *et al.,* 2017).

Food fats are assumed to be the primary source of energy, however total fats also contain unhealthy saturated and trans fatty acids. Low fats foods are therefore preferred for human consumption. The fat content in our samples was comparably low to the values obtained by Belewu *et al.* (2009) for *Ocimum gratissimum* (11.75%) and *Vernonia amygdalina* (13.40%). But the values recorded are comparable to several previous findings (Ejoh *et al.,* 2007; Satter *et al.,* 2016). The low fat content of the samples studied suggests the plant may used as a low fat food for individuals on weight-reducing diets (Emebu and Anyika, 2011; Brahma *et al.*, 2014).

According to Tuncturk and Ozgokce (2015), a plant's ash level is a good predictor of its overall mineral richness. The mineral contents present in the food materials is directly related to the quantity of ash content (Omotoso, 2006). Total ash content obtained in our study corresponds to the findings of Seal and Chaudhuri, 2016; Satter *et al.* (2016) for some wild vegetables; Aletan and Kwazo (2019); Olumide *et al.* (2019) for ash contents of wild edible plants. The values obtained in this study were much higher than that obtained by Belewu *et al.* (2009) for *Ocimum gratissimum* (3.58%) and *Vernonia amygdalina* (4.85%) and by Otunola *et al.* (2020) for *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl. leaves. The total ash values obtained were also high comparatively to the range of 5.43-5.75% reported for some edible woody plants (Emmanuel *et al.*, 2011) and the range of 0.38-1.9% for selected vegetables grown in Peshawar (Bangash *et al.*, 2011). The ash content of *Triumfetta rhomboidea* leave sample (3.38±0.22%) reported by Akintimehin *et al.* (2022) is also low as compared to our samples. The values of ash observed in all the leaves are an indicator that these samples are good sources of minerals (FAO, 1980). The Institute of Medicine (IOM) set the recommended dietary allowances (RDA) for dietary fibre for adults to 25gm per day in its 2002 report. Haub and Lattimer, (2010) reported that the presence of high fibre lowers cholesterol level in the blood, reduces the risk of various cancers, bowel disease and improves general health and well being. The fibre content obtained in the present study can be comparable to the findings of Satter *et al.* (2016) in some wild vegetables and Akintimehin *et al.* (2022) in *Triumfetta rhomboidea* leave sample. The fibre contents of our samples are high as compared to the many previous report (Sun *et al.,* 2018; Olumide *et al.,* 2019; Aletan and Kwazo, 2019; Tharmabalan, 2021).

Carbohydrate is an essential class of food that is stored in the body, serves as precursor for biological synthesis of many compounds and provides energy for living systems (Islary *et al.,* 2016). The carbohydrate content recorded in the present study is higher than the values obtained by *Clerodendrum colebrookianum* (4.28±1.08 %) by Payum (2020). But the carbohydrate content detected in all our samples was below the RDA values established by IOM in its 2002 reports. Islary *et al.* (2016) reported the carbohydrate content (i.e., 80.18±0.02%) in *Grewia sapida* fruit freeze dried sample which is much higher than our values obtained. Food with low carbohydrate content is considered ideal for diabetic and hypertensive patients requiring low-sugar diets (Singha and Hassan, 2017). The calculated energy value obtained of our sample is less than the energy of 2500-3000 kcal required for adults (WHO/FAO, 1985). Because of the low calorie value of our sample leaves, they are suitable for weight loss and for persons suffering from obesity (Ullah *et al.,* 2013).

Proximate components in food are essential for the normal growth and development of a healthy body and secondary metabolites included in diet act as nutraceuticals, aiding in the treatment of a variety of health conditions. The nutritional composition of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* leaf was discovered in this study. Because of their high nutritional value, the selected plant species could be used for human nutrition, providing adequate protection against diseases induced by malnutrition. It also emphasizes the importance of edible ethno medicinally important plants as low-cost sources of nutrition for rural tribals. As a result, it can be stated that frequent eating of these plants can help to alleviate malnutrition and cure dangerous diseases.

Minerals also serve an important role in the human body's healthy function and wellness. Inadequate mineral consumption in the diet is frequently connected to an increased vulnerability to viral diseases due to immune system weakness (Sajib *et al.,* 2014). Minerals, even in trace amounts, play an important role in plant healing. The daily requirement for trace or micro minerals for an adult is less than 50mg (Liz, 2006). Medicinal plants have also been linked to heavy metal poisoning in both people and animals (Dwivedi and Dey, 2002). Heavy metals such as Cadmium, Lead, Chromium, Mercury and Nickel are toxic and must be monitored (Altundag and Tuzen, 2011). All the three candidates' plants tested negative for heavy metals during heavy metal detection. The current study's results were within the permissible levels set by the Food and Nutrition Board, Institute of Medicine, US, demonstrating that these ethno medicinally important plants provide a positive contribution of mineral elements to the diets of consumers. Among the analyzed minerals, Potassium content was the highest at about 104.7 ± 0.20 mg/100g detected in *Z. oxyphyllum* followed by calcium (66.37 ± 0.06 mg/100g) in *B. lanceolaria*.

The Potassium content detected in *Z. oxyphyllum* is found to be high than that reported by Khalil *et al.* (2010); Alagbe *et al.* (2020) in *Delonix regia* leaves and roots. Das *et al.* (2023) reported the K content for *Basella alba* L., *Basella rubra* L., *Rosa indica* L., *Rosa damascene* Mill. and *Rosa bracteata* L. which is lower than our findings. Sodium content detected in our samples is comparable to the content reported by Das *et al.* (2023) for *Basella rubra* L. But are higher than *Rosa indica* L., *Rosa damascene* Mill and *Rosa bracteata* L. Sodium and Potassium are two essential macro minerals that the body need to sustain cellular homeostasis, metabolism and a variety of other processes. Our bodies' Na/K ratio is crucial for managing high blood pressure and should be less than one (Akubugwo *et al.,* 2007). According to the literature, Sodium enhances blood pressure while Potassium lowers blood pressure (Saupi *et al.,* 2009). In our investigation, all of the samples had a Na/K ratio smaller than one, indicating that consumption of all these three edible medicinal plants under study would probably reduce high blood pressure diseases because the ratio of Na/K in all the plants was less than one. Na/K ratio less than one is recommended as mentioned by Akubugwo *et al.* (2007). Low Sodium and high Potassium intake also aid in the prevention of hypertension and atherosclerosis (Saupi *et al.,* 2009).

The role of Magnesium and Calcium in maintenance of heart function has been well pointed out by Insel *et al.* (2010). Calcium has also been shown to aid in the formation of skeletal structures and muscle function, whereas magnesium, as a macro-element, aids in ionic equilibrium and enzyme co-factors (Arunachalam and Parimelazhagan, 2014). Magnesium works with calcium to help with muscle contraction and blood clotting (Gnansounou *et al.,* 2014). In our study, high concentration of Magnesium was found in *R. serrata* as compared to *Z. oxyphyllum* and *B. lanceolaria*. These results are in agreement with the findings of Anyasor *et al.* (2014) in *Costus afer* leaf and stem. Seal and Chaudhuri, (2016) recorded Mg content for *Gnetum gnemon*, *Polyrhachis hookeri*, *Blumea lanceolaria* and *Pilea melastomoides* which is lower than our findings. According to the results of our investigation, the percentage fulfillment of Calcium is found to be high among macro minerals in all samples, with the highest value recorded in *B. lanceolaria* (6.63%), followed by *R. serrata* (6.27%) and *Z. oxyphyllum* (4.13%).

According to the findings, these edible plants could be a useful source of calcium in our diet, as well as providing health benefits by lessening the risk of such diseases. The Calcium content in our study is higher than the reported result of Bakari *et al.* (2017) for *Opuntia ficus-indica* var. inermis and Das *et al.* (2023) for *Basella alba* L., *Basella rubra* L., *Rosa indica* L., *Rosa damascene* Mill. and *Rosa bracteata* L., reported (Uraku, 2017; Labaran *et al.,* 2016) for *Culcasia scandens* leaf (10.80 mg/100 g), *Albizia lebbeck* leaf (16.0 mg/100g). Khalil *et al.*

(2010) reported the Calcium content for *Petroselinum crispum* (42.9mg/100g) and *Corianderum sativum* (64.8 mg/100g) are comparable to our findings in *Z. oxyphyllum* (41.38 ± 0.01mg/100g) and *R. serrata* (62.73 ± 0.20 mg/100g) and *B. lanceolaria* (66.37 ± 0.06 mg/100g). The Calcium content in *R. serrata* and *B. lanceolaria* is comparable to the previous finding of Andualem and Gessesse, (2014) in Brebra seed. Singa, (2018) reported the Calcium content in *Celosia argenta* and *Alternanthera sessilis* which is lower than our study.

Despite being the least abundant micronutrient in the samples, Manganese had the highest RDA fulfillment percent. Manganese is an essential component of metalloenzymes and plays an important role in a variety of physiologic processes as a constituent or activator of certain enzymes required for glucose, cholesterol, and amino acid metabolism (Pandey *et al.,* 2012). Mn content of *Z. oxyphyllum* in our study is found comparable with the findings of Khalil *et al.* (2010) in *Corianderum sativum* as mentioned. But the Manganese content in *R. serrata* and *B. lanceolaria* is higher than the reported findings of Khalil *et al.* (2010). Zinc is an essential nutrient for human growth and resistance to infection (Islam *et al.,* 2015). It functions as a cofactor for the antioxidant enzyme superoxide dismutase and is essential for the activity of over 300 different enzymes as well as a number of enzymatic activities involved in carbohydrate and protein metabolism (Aberoumand and Deokule, 2009). Excessive intake of zinc has been reported to be toxic (Salama and Radwan, 2005). The recommended dietary allowance for zinc is 8 mg per day for adult women and 11mg per day for adult men which appear to be sufficient to prevent deficiency in most individuals (Sajib *et al.,* 2014). Manganese and Zinc concentration in all samples was found to be lower than the acceptable limit by analysis. Demirezen and Ahmet (2006), analyzed various vegetables and reported that the Zinc concentrations in vegetables are within the recommended international standards. Satter *et al.* (2016) reported Zinc content detected in some wild vegetables in Bangladesh which are comparable with our findings. But the Zinc content detected is higher than the results reported by Khalil *et al.* (2010) for some medicinal plants and Alagbe *et al.* (2020) in *Delonix regia* leaves and roots. The Zn detected in *R. serrata* is in agreement with the finding of Otunola *et al.* (2021) in *Heteromorpha arborescens* leaves. But it is higher in *Z. oxyphyllum* and *B. lanceolaria* as reported by Otunola *et al.* (2021).

In the earlier study as reported by Obazelu *et al.* (2021), it was found that the Calcium, Sodium, Zinc, Manganese content detected in *Combretum platypterum* leaf is lower than our samples. Iron is an important trace element in the human body. It plays a vital role in haemopoiesis, control of infection and cell mediated immunity (Bhaskaram, 2001; Kozat, 2007). Iron deficiency anemia is estimated to affect more than one billion people worldwide (Trowbridge and Martorell, 2002). All analyzed samples possess an adequate amount of Iron. The percentage of RDA recorded shows that *R. serrata* has the highest percent fulfilment in comparison to *Z. oxyphyllum* and *B. lanceolaria* in male. But for female, the highest recommended fulfilment percentage was found in *R. serrata.* Iron content found in our investigated samples is comparable to the findings of Khalil *et al.* (2010) in some medicinal plants and Das *et al.* (2023). The Iron content in *Z. oxyphyllum* and *R. serrata* is higher than as reported by *Delonix regia* leaves and roots (Alagbe *et al.,* 2020). The Iron content in *B. lanceolaria* is also comparable to the findings of Bakari *et al.* (2017) in cladodes powder and Alagbe *et al.* (2020) in *Delonix regia* leaves and roots. But it is lower than the Iron content in *Z. oxyphyllum* and *R. serrata*.

Copper deficiency has been reported to cause cardiovascular disorders as well as anaemia (Olumide *et al.,* 2019). It is an essential trace element in human body has an important role in oxidation reduction reactions and in scavenging of free radicals (Linder and Azam, 1996). When its concentration exceeds the safe limit, it can be toxic in some cases (Ogwok *et al.,* 2014). Among the analyzed samples, the percent fulfillment of recommended intake for Copper was found high in *B. lanceolaria.* The Copper content detected in *B. lanceolaria* is in agreement with Copper content of Helencha and Shapla stem by Satter *et al.* (2016). The Copper in *B. lanceolaria* is higher than reported by Otunola *et al.* (2020) in *Heteromorpha arborescens* leaves and Obazelu *et al.* (2021) in *Combretum platypterum* leaf. The Copper content in Helencha and Shapla stem as reported by Satter *et al*. (2016) which supports our results. A trace quantity of Nickel may promote healthy skin, iron metabolism and optimal growth in humans, but when its concentration exceeds the safe limit it can be toxic (Satter *et al.,* 2016). The RDA percentage recorded for Nickel was found high in *B. lanceolaria*. Chromium the toxic heavy metal detected in *Z. oxyphyllum* was within permitted limit of RDA. According to Satter *et al*. (2016), the Chromium level of various vegetables exceeded the allowable limit. Chromium was also found to be higher than previously reported levels for various wild green leafy vegetables from Northern East India (Saikia and Deka, 2013). These high amounts of Chromium in the vegetables could be attributed due to contamination in the soil, wastewater or industrial effluents (Ramesh and Yogananda, 2012). The low level of Chromium content in *Z. oxyphyllum* and the absence of Chromium in *B. lanceolaria* and *R. serrata* indicate that they are safe and devoid of Chromium contamination.

AMINO ACID ANALYSIS

The concentration of amino acids was determined by comparing the peak area of the sample to that of the standards. Percentages of total amino acid in each plant were also calculated. Both essential and non essential amino acids were discovered to be present in varied amounts in the samples. The amino acid profile compared with the FAO/WHO/UNU (1985) consultation pattern of requirement for a 2-5 year preschool child.

Among the investigated samples, all the essential amino acid was observed in *B. lanceolaria* while the other two viz., *Z. oxyphyllum* and *R. serrata* were found to be lacking in either one or three essential amino acid. Among the essential amino acid, histidine was absent in both *Z. oxyphyllum* and *R. serrata*. Both threonine and methionine was found absent in *R. serrata*. The values of phenylalanine were found to be high for all samples. Of the non essential amino acid, cystein was absent in all the samples. The amino acid score of *Z. oxyphyllum* and *B. lanceolaria* was 42% and 64%, methionine and leucine was the limiting amino acid respectively. While valine with an amino acid scores of 57 % in *R. serrata* was found to be the limiting amino acid. The ratio between the non essential and essential amino acids in the investigated samples ranged from 1.09 - 1.81. The total EAA ranged from 35.5% to 52.14% with the highest amount was that of *B. lanceolaria* followed by *Z. oxyphyllum* and *R. serrata.* The result indicated that *B. lanceolaria* were rich in essential amino acid as compared to *Z. oxyphyllum* and *R. serrata.* The total NEAA ranged from 47.75% to 64.44 % with the highest amount was that of *R. serrata* followed by *Z. oxyphyllum* and *B. lanceolaria*. This indicated that *R. serrata* has maximum amount of non- essential amino acid.

Amino acid composition analysis provides details about the protein content of foods. They are precursors for the secondary metabolites synthesis that have physiological beneficial effects in our bodies. They are essential components for healing processes and a lack of these components impedes recovery. Aside from their structural roles, amino acids are the primary building blocks for the synthesis of a variety of vital compounds in the bodies of living things. They may also be crucial sources of energy particularly when the body is deficient in carbohydrates and fats in the body (Olusanya, 2008). In the present investigation, essential amino acid threonine was detected only in *Z. oxyphyllum* (2.90 ± 0.02mg/100g) and *B. lanceolaria* (0.64 ± 0.01mg/100g). Threonine was not detected in *R. serrata* which supports the findings of Jaimes-Morales *et al.* (2022) who reported the absence of threonine in *Prosopis juliflora* seed. Olayinka *et al.* (2023) reported the threonine content in eight cultivars of ground nut which is comparable to our findings of *Z. oxyphyllum.* Similarly, Sibiya *et al.* (2021) reported the threonine content in *Mimusops zeyheri* seed which is lower than our results. The threonine content in *Z. oxyphyllum* (2.90 ± 0.02mg/100g) is higher as compared to Zhao *et al.* (2018) in dried Laver (Porphyra spp.) seaweed (1.52 ± 0.02mg/100g). The threonine content detected in *Z. oxyphyllum* and *B. lanceolaria* is also found high with the reported results of Leea and Finnb, (2012) in five cultivars of Lingonberry (Vaccinium vitis-idaea L.). Mohanty *et al.*, (2014) reported the threonine is an essential amino acid for the treatment of disorders in nervous system including multiple sclerosis, spinal spasticity, amyotrophic lateral sclerosis and familial spastic paraparesis.

Other amino acids, such as valine, are essential for maintaining mental acuity and muscle coordination; they also regulate the proportion of branched-chain amino acids (Oguoma *et al.*, 2015). Valine content was found present in all samples of which *Z. oxyphyllum* has the highest valine content (2.57 ± 0.02mg/100g) followed by *B. lanceolaria* (0.66 ± 0.02mg/100g) and *R. serrata* (0.19 ± 0.01mg/100g). Sodamade *et al.* (2021) reported the valine content in *Mormodica charantia* which is lower than *Z. oxyphyllum*. The valine content detected in *Z. oxyphyllum* and *B. lanceolaria* are higher than *Mimusops zeyheri* seed as mentioned by Sibiya *et al.* (2021). The Valine content in *Z. oxyphyllum* is comparable to *Hibiscus syriacus* roots (2.4 ± 0.1mg/100g) as reported by Park *et al.* (2022). The valine content in *B. lancealaria* is comparable to Sanna cultivars of Lingonberry (Vaccinium vitis-idaea L.) as reported by (Leea and Finn, 2012) but in *Z. oxyphyllum*, the valine content is higher. Valine and leucine exhibit anti-inflammatory activities and together stimulate the muscle growth (Wu, 2009) and moreover, their supplementation is common in liver cirrhosis patients (Holecek, 2017).

Methionine detected in *Z. oxyphyllum* (0.59±0.01mg/100g) and *B. lanceolaria* (0.70 ± 0.01mg/100g) in our study. These studies are comparable to the findings of Olayinka *et al.* (2023) detected in eight cultivars of ground nut. Kalidass and Mohan (2011), also reported the methionine content of *Mucuna pruriens* var. pruriens in Ayyanarkovil Reserve Forest Virudhunagar Dist, Tamil Nadu and Aliyar Reserve Forest, Coimbatore Dist, Tamil Nadu which is also similar to our findings in *Z. oxyphyllum* and *B. lanceolaria*. Methionine content in our samples is also higher than *Mimusops zeyheri* seed as reported by Sibiya *et al.* (2021). Park *et al.* (2022) reported the methionine content in *Hibiscus syriacus* leaves (0.5 ± 0.1mg/100g), Roots (0.1±0.0 mg/100g) and sprouts (0.4±0.1mg/100g) which is lower as compared to our result of *Z. oxyphyllum* and *B. lanceolaria.* The methionine content in *Hibiscus syriacus* leaves (0.5 ± 0.1mg/100g) is comparable to *Z. oxyphyllum* (0.5 ±0.1mg/100g). Methionine is imperative in mammalian nutrition as its supplementation, or restriction can mediate the natural antioxidant capacity of an organism by the formation of endogenous enzymes that reduce oxidative stress, DNA damage, cancer, cardiovascular, and neurodegenerative diseases (Martinez *et al.*, 2017). Methionine can be a potential component of artificial feeding formulas and for the prevention of breast and colorectal cancer (Van de *et al.*, 2006). It promotes the detoxification of harmful agents such as lead and other heavy metals. Methionine can improve damaged heart function. Isoleucine, which is an isomer of leucine does not have itself the ability to stimulate insulin synthesis, but in combination with leucine, their secretolytic activity increases significantly, causing a more pronounced hypoglycaemic effect (Comerford and Pasin, 2016; Birech *et al.*, 2017).

Park *et al.* (2022) reported the isoleucine content in *Hibiscus syriacus* root which is comparable to our findings in *Z. oxyphyllum*. The Isoleucine content in *Z. oxyphyllum* and *B. lanceolaria* is found higher as compared to the reported result of Sibiya *et al.* (2021) in *Mimusops zeyheri* seed (0.38mg/100g) and Zhao *et al.* (2018) in dried Laver (Porphyra spp.) seaweed (0.39 ± 0.11mg/100g). The Isoleucine content in five cultivars of Lingonberry (Vaccinium vitis-idaea L.) as reported by (Leea and Finn, 2012) is lower than our findings of *Z. oxyphyllum* and *B. lanceolaria*. But the isoleucine content in *R. serrata* is comparable. Leucine is present in all our samples studied in varied amount. The highest amount of leucine was present in *Z. oxyphyllum*. Leucine content in our samples is found higher than the findings of Sibiya *et al.* (2021) in *Mimusops zeyheri* seed. Park *et al.* (2022) and Zhao *et al.* (2018) reported the leucine content in *Hibiscus syriacus* root and in dried Laver (Porphyra spp.) seaweed respectively supports our findings of *B. lanceolaria*. But the leucine content in *Z. oxyphyllum* is found high as compared to the reported result of Zhao *et al.* (2018). The leucine in our samples is higher than that reported by Leea and Finn, (2012) in five cultivars of Lingonberry (Vaccinium vitis-idaea L.).

Phenylalanine is the precursor of some hormones and the pigment melanin in hair, eyes and tanned skin (Quadri and Musa, 2015). It participates in the formation of collagen in the body, improves memory, attention and mood. It is also essential for immune function. Among the three samples, the maximum amount of phenylalanine was found *Z. oxyphyllum* (7.08 ± 0.02mg/100g) followed by *B. lanceolaria* (5.77 ± 0.03mg/100g) and *R. serrata* (1.76 ± 0.03 mg/100g). Leea and Finn, (2012) reported the phenylalanine content in five cultivars of Lingonberry (Vaccinium vitis-idaea L.) which is lower than ours findings. The phenylalanine content of our samples supports the findings of Park *et al.* (2022) in *Hibiscus syriacus* leaves, roots and sprouts. Olayinka *et al.* (2023) also reported the comparable phenylalanine content of ground nut with *B. lanceolaria*. But the phenylalanine content in *Z. oxyphyllum* is higher than the reported results of Sodamade *et al.* (2021) in *Mormodica charantia*. Kalidass and Mohan (2011) also determined the amino acid composition of five accessions of *Mucuna pruriens* var. pruriens where the phenylalanine content is lower than our results of *Z. oxyphyllum* and *B. lanceolaria*. Phenylalanine content in *Z. oxyphyllum* and *B. lanceolaria* is also found higher than *Diospyros mespiliformis* and *Mimusops zeyheri* seed by Sibiya *et al.* (2021) and in dried Laver (Porphyra spp.) seaweed as reported by Zhao *et al*. (2018). Similarly, the phenylalanine content in *R. serrata* is found high than *Mimusops zeyheri* seed as reported by Sibiya *et al.* (2021). The phenylalanine content in *Z. oxyphyllum* supports the findings of Thushar *et al.* (2023). Budniak *et al.* (2022) reported the phenylalanine content in *Angelica archangelica* L. which is lower than our result in *Z. oxyphyllum*. The phenylalanine content in *R. serrata* is comparable to Zhao *et al.* (2018) in dried Laver (Porphyra spp.) seaweed (1.11mg/100g).

Histidine bestows anti-inflammatory properties and is an efficient scavenger of free radicals and plays an antioxidative role in muscle, brain and other tissues (Hasegawa *et al.*, 2012). Histidine is used in the treatment of cardiovascular disease with a physiological antioxidant role it plays on the free radicals (hydroxyl radical and singlet oxygen). In the present study, histidine was present only in *B. lanceolaria* (0.28 ± 0.005 mg/100g). Chibueze and Peters (2022), also reported the absence of histidine in *Costus lucanusianus* stem.

Lysine is helpful in the absorption and production of calcium; it is required for protein synthesis hormone and energy production. The Lysine content in *Z. oxyphyllum* and *B. lanceolaria* is higher as compared to the reported result of Sibiya *et al.* (2021) in *Mimusops zeyheri* seed. The Lysine content in *Z. oxyphyllum* is also high than *Hibiscus syriacus* root (Park *et al.*, 2022) and Zhae *et al.* (2018) in dried Laver (Porphyra spp.) seaweed. Jaimes-Morales *et al.* (2022) reported the lysine content in *Prosopis juliflora* seed which is comparable to our *Z. oxyphyllum*.

The lysine content in our samples is higher than that reported by Leea and Finn, (2012) in five cultivars of Lingonberry (Vaccinium vitis-idaea L.). Arginine, an non-essential amino acid exert influence on blood pressure and its supplementation depicts glucoregulatory and insulinotropic effects in diabetic patients (Krause *et al.*, 2017; Newsholme *et al.*, 2017). Oni *et al.* (2021) also reported that arginine has numerous functions including the treatment of chest pain, high blood pressure and pregnancy complications such as pre-eclampsia as well as erectile dysfunction. Additionally, it enhances the body's defense responses to tumor cells, bacteria and viral infections.

Arginine was detected in all the samples in our present investigations. The highest arginine content was detected in *Z. oxyphyllum* followed by *B. lanceolaria* and *R. serrata*. Arginine content in *Z. oxyphyllum* in our investigation is comparable to the reported results of Olayinka *et al.* (2023). Sodamade *et al.* (2021) reported the arginine in *Mormodica charantia* which is lower than our *Z. oxyphyllum*. Kalidass and Mohan (2011) also determined the amino acid composition of five accessions of *Mucuna pruriens* var. pruriens. Ariginine content detected in *Z. oxyphyllum* is higher than the reported result of Kalidass and Mohan (2011). The arginine content detected in *Z. oxyphyllum* is comparable with the reported results of Leea and Finnb, (2012) in five cultivars of Lingonberry (Vaccinium vitis-idaea L.). Other non-essential amino acid asparagine content in our study is found highest in *Z. oxyphyllum* (2.37 ± 0.02 mg/100g) and *B. lanceolaria* (2.21 ± 0.03 mg/100g). *R. serrata* has the asparagines content of (0.28 ± 0.01 mg/100g). Budniak *et al.* (2022) reported the asparagines content in *Angelica archangelica* L. which supports our findings in *Z. oxyphyllum* and *B. lanceolaria*.

Serine is necessary for the muscles' development and the immune system's maintenance. It acts as precursor for the synthesis of glycine, cysteine and tryptophan. It participates in cell signalling and also helps in treatment of Schizophrenia (Mohanty *et al.*, 2014). *Z. oxyphyllum* has the highest serine content (3.02 ± 0.01 mg/100g) in comparison to *R. serrata* (0.83 ± 0.02 mg/100g) and *B. lanceolaria* (0.28 ± 0.005 mg/100g). Zhao *et al.* (2018) reported the serine content in dried Laver (Porphyra spp.) seaweed (0.13 ± 0.02 mg/100g) which is much lower than our findings. The serine content in *Z. oxyphyllum* is comparable to the findings of Sodamade *et al.* (2021) in *Mormodica charantia*. Mohan and Kalidas (2011), reported the amino acid composition of five accessions of *Mucuna pruriens* var. pruriens. The serine content of *Mucuna pruriens* var. pruriens collected from Aliyar Aliyar Reserve Forest, Coimbatore Dist, Tamil Nadu. and Seithur Reserve Forest, Virudhunagar Dist, Tamil Nadu is in accordance with our result of *Z. oxyphyllum*. Leea and Finnb, (2012) determined amino acid composition of five cultivars of Lingonberry (Vaccinium vitis-idaea L.). Serine content in *Z. oxyphyllum* is comparable to the findings of Leea and Finnb, (2012).

Glutamine is most abundant in the muscle and its presence allows for the building and maintenance of muscle tissue (Abdou Bouba *et al.,* 2016). The glutamine content of our study is similar with the reported results of Leea and Finnb, (2012) in Sussi cultivars of Lingonberry (Vaccinium vitis-idaea L.). Proline non-essential amino acids that exhibits significant hypoglycaemic activity which is due to a decrease in hepatic glucose production owing to inhibition of glycogenolysis, gluconeogenesis and glucose-6-phosphatase activity (Chen *et al.* 2010). Zhao *et al.* (2018) reported the proline content in dried Laver (Porphyra spp.) seaweed (0.13 ± 0.02 mg/100g) which is much lower than our findings in plant samples. The proline content in *Z. oxyphyllum* is higher than that reported by Leea and Finnb, (2012) in five cultivars of Lingonberry (Vaccinium vitis-idaea L.). Mohan and Kalidas (2011), investigated the amino acid composition of five accessions of *Mucuna pruriens* var. pruriens. The proline content of *Mucuna pruriens* var. pruriens is lower than our findings of *Z. oxyphyllum*. The proline content in *Z. oxyphyllum* is higher than the many reported results of Olayinka *et al.* (2023) in ground nut and of Park *et al.* (2022) in *Hibiscus syriacus* leaves and Sodamade *et al.* (2021) in *Mormodica charantia*. Thushar *et al.* (2023) reported the proline content in *Zygophyllum qatarense* Hadidi and *Salsola imbricata* Forssk. is comparable to the finding in *Z. oxyphyllum.*

Proline is the main component of collagen and in the presence of vitamin C helps wound healing, promotes good joint function, participates in the formation of biologically active peptides and can be used in the treatment of injuries. Glycine and his derivatives act as hypolipidemic agents; some amino acids stimulate insulin secretion by pancreatic b-cells (Mark *et al.*, 2018). Glycine participates in the synthesis of glutathione increasing the antioxidant capacity of the plant. The highest glycine content detected in *Z. oxyphyllum* (5.37± 0.03mg/100g) compared to *R. serrata* and *B. lanceolaria*. The glycine content detected in *Z. oxyphyllum* supports the findings of Mohan and Kalidas (2011) in Seithur Reserve Forest, Virudhunagar Dist, Tamil Nadu. Olayinka *et al.* (2023) reported the glycine content in ground nut (5.54 mg/100g) which supports our finding in *Z. oxyphyllum*. Park *et al.* (2022) reported the glycine content in *Hibiscus syriacus* roots (2.2 ± 0.1mg/100g) and in *Mormodica charantia* by Sodamade *et al.* (2021) which is lower than our data in *Z. oxyphyllum* (5.37 ± 0.03mg/100g). Similary, Zhae *et al.* (2018) reported the glycine content in glycine content in dried Laver (Porphyra spp.) seaweed which is found low than *Z. oxyphyllum*. *Zygophyllum qatarense* Hadidi. reported by Thushar *et al.* (2023) is comparable to the finding in *Z. oxyphyllum.* The glycine content of our study is found high with the reported results of Leea and Finnb, (2012) in five cultivars of Lingonberry (Vaccinium vitis-idaea L.). Cystein is not detected in all the plant samples. Ahmed *et al.* (2017) also reported the absence of cystein in the stems of *Ficus cordata* thunb. Subsp. Salicifolia.

Alanine plays a crucial role in autophagy, gluconeogenesis and transamination processes in the liver (Abdou Bouba *et al.*, 2016). The alanine content in *Z. oxyphyllum* is comparable to the findings of Olayinka *et al.* (2023) and Park *et al.* (2022) in *Hibiscus syriacus* roots. Mohan and Kalidas (2011), reported the alanine content of *Mucuna pruriens* var. pruriens in Seithur Reserve Forest, Virudhunagar Dist, Tamil Nadu which is close to our findings in *Z. oxyphyllum*. The alanine content detected in *Z. oxyphyllum* is found higher as compared to the reported results of Leea and Finnb, (2012) in five cultivars of Lingonberry (Vaccinium vitis-idaea L.).

Tyrosine and phenylalanine are the primary metabolites that serve as precursors for flavonoids. Among the analyzed plants, the tyrosine content in *Z. oxyphyllum* (4.49 ± 0.01mg/100g) than *B. lanceolaria* (1.85 ± 0.03 mg/100g) and *R.*

serrata (0.64 ± 0.02 mg/100g). The tyrosine content in *Z. oxyphyllum* (4.49 ± 0.01mg/100g) is higher than the findings of Olayinka *et al.* (2023) and Sodamade *et al.* (2021) in *Mormodica charantia*. The tyrosine content in *Z. oxyphyllum* supports the data obtained by Moteetee *et al.* (2021) in *Azanza garckeana* and Kalidass and Mohan, (2011) of *Mucuna pruriens* var. pruriens in Ayyanarkoil Reserve Forest, Virudhunagar Dist, Tamil Nadu. But the tyrosine content of *Z. oxyphyllum* is higher than Sivagiri, Anaikatti and Seithur Reserve Forest, Tamil Nadu. The tyrosine content in *B. lanceolaria* is higher than the content in *Mimusops zeyheri* seed. Park *et al.* (2022) reported the tyrosine content in *Hibiscus syriacus* leaves (2.7 ± 0.1 mg/100g), petals (3.2 ± 0.1 mg/100g) and sprouts (1.6 ± 0.2 mg/100g) which is lower than *Z. oxyphyllum*. The tyrosine content in *B. lanceolaria* is comparable to *Hibiscus syriacus* sprouts as reported by Park *et al.* (2022). The tyrosine content in *Z. oxyphyllum* is comparable to the findings of Zhao *et al.* (2018) in dried Laver (Porphyra spp.) seaweed. The tyrosine content of *R. serrata* is similar to the finding of Leea and Finnb, (2012) in Sanna cultivars of Lingonberry (Vaccinium vitis-idaea L.). The tyrosine content in *B. lanceolaria* supports the data of Leea and Finnb, (2012) in Ida and Linnea cultivars of Lingonberry (Vaccinium vitis-idaea L.). But the tyrosine content of *Z. oxyphyllum* is higher than all five cultivars of Lingonberry (Vaccinium vitis-idaea L.).

Our findings have clearly shown the presence of most essential and non essential amino acids indicating that the studied plants may possibly contribute in mitigating protein deficiency. Additionally, it raises the prospect of incorporation the studied plant material into the modern health care system through providing useful information for further application of these plants. The findings of the present study were compared with the available reports. It seems that the results obtained during the present investigation were comparable with the results reported by various workers.

FATTY ACID ANALYSIS

Fatty acids are significant bioactive substances that participate in intricate metabolic processes and play vital functions in biology. When evaluating the nutritional value of food, fatty acids have a major biological role. Fatty acids are also very important in pharmacology and illness diagnosis (Stoddart *et al.,* 2008). Fatty acids can be derived and used from various sources, including numerous medicinal plants, and are generally valued and reasonably priced. They also have a wide range of medical capabilities, including anti-fungal and anti-bacterial qualities. Fatty acids occur naturally in mixtures of saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), thus their nutritional and/or therapeutic properties must be identified. FAs are transported to cells, where they serve as fuel for muscle contraction and overall metabolism. FAs are biological molecules that play important roles in human metabolism, health and illness.

Epidemiological research and clinical trials revealed that fatty acids are related with cardiovascular illnesses (Rimm *et al.,* 2018; Wu *et al.,* 2020; Marangoni *et al.,* 2020; Naeini *et al.,* 2020; Bird *et al.,* 2018). neurological diseases (Tomata *et al.,* 2020; Langley *et al.,* 2020; Zhou *et al.,* 2019; Chang *et al.,* 2020), non-alcoholic fatty liver disease (Konstantynowicz-Nowicka *et al.,* 2019; Chen *et al.,* 2018; Chen *et al.,* 2019; Wu *et al.,* 2020), allergic diseases (Tobias *et al.,* 2019; Monga *et al.,* 2020; Magnusson *et al.,* 2018) and so on. FAs can have an impact on illness prevention and therapy, either positively or negatively. For example, SFAs may increase the chance of developing multiple sclerosis (MS) and disease progression, but PUFAs may benefit MS patients (Langley *et al.,* 2020). For example, whereas some important FA metabolites may have health benefits such as anti-inflammatory and neuroprotective properties, they can also cause inflammation, necrosis promotion and atherosclerosis.

In general, FAs are derived from a variety of dietary sources that have distinct FA compositions and so influence health outcomes. From this perspective, the FA composition should be evaluated in order to establish their nutritional and/or medical value, particularly in fatty-acid-rich meals, food supplements and herbal-based medications. In the sample under analysis, both saturated and unsaturated fatty acids were discovered. The saturated fatty acids provide energy to the human body. They are involved in the transport and absorption of vitamins and trace elements, hormone synthesis and cell membrane formation. Unsaturated fatty acids also play a significant role in the body's essential activities. Unsaturated fatty acids from the omega-3, omega-6 and omega-9 classes play an important role in the human body, including providing cell membrane function, exhibiting anticholesterolemic activity, converting cholesterol into cholic acids and removing them from the body, and participating in fat metabolism (Sears and Perry 2015; Brown *et al.*, 2019). Unsaturated (monounsaturated or polyunsaturated) fatty acids are commonly utilized to reduce the risk of heart disease, inflammation, and improve immunity (Calder 1999). Furthermore, they have anti-inflammatory properties via synthesizing prostaglandins and exhibiting immunomodulatory activity. They also improve blood circulation and nervous system function. All of these fatty acid characteristics are critical for treating diabetic patients and preventing problems such as diabetic micro- and macroangiopathies (Li *et al.*, 2018; Rogero and Calder, 2018).

It was discovered that the studied plant leaves contain fatty acids (palmitic, stearic and oleic acid, with the latter being the predominant component). The fatty acids found in the samples, particularly palmitic and stearic acid, are employed in the pharmaceutical and cosmetic industries (Kalustian, 1985). Palmitic acid is utilized in skin care products as an emulsifier in the form of alkali salts like sodium or potassium palmitate. Oleic acid (Omega-9-fatty acid), monounsaturated fatty acid identified in *B. lanceolaria* is reported for antioxidant, antimicrobial, anti-inflammatory, anti-ulcer, anticancer, anti-tuberculosis, anti-obesity, anti diarrheic and antibacterial activity (Ramya *et al.*, 2015; Ralte *et al.*, 2022). According to literature reviews, the fatty acid makeup of specific medicinal plants has not before been investigated. This is the first research containing fatty acid profiles for plant species.

ANTI-NUTRITIONAL STUDY

In the present study, the oxalate content is highest in the leaves of *B. lanceolaria* (11.04±0.06 mg/100g) followed by *Z. oxyphyllum* (7.99±0.02mg/100g) and *R. serrata* leaves (7.96±0.13mg/100g). The oxalate content detected in the present study is lower than the oxalate contents of some selected wild edible plants namely *Erucastrum abyssinicum* (11.73± 0.25mg /100g) and *Haplocarpha rueppelii* (9.09 ± 0.25mg/100g) as reported by Adamu *et al.* (2022). Kiliobas *et al.* (2019) also detected the oxalate content of *Corchorus oliterius* plant which is much higher than the present study. The oxalate content detected in some wild edible plants consumed in South East Ethopia by Yimer *et al.* (2023) which is much higher than our findings.

Engtipi and Raju, (2022) reported the anti nutritional content of five indigenous spices used by the Karbi Group of Assam is comparable to our findings. Oxalate is a supplement antagonist that binds to minerals in the gastrointestinal tract, resulting in mineral shortage in the body (Ilelaboye *et al.,* 2013; Muhammed *et al.,* 2002). High levels of oxalic acid in meals can interact with components in the gastrointestinal tract to generate oxalates which are responsible for the production of calcium oxalate stones in the kidney (Bele *et al.,* 2010). Oxalate has been shown to impair renal calcium absorption especially at centralizations of 45g/100g (Muhammed *et al.,* 2002).

According to Gemede and Ratta (2014), the presence of oxalate in foods or vegetables at levels above acceptable causes irritation in the mouth and the lining of the gut as well as a reduction in the absorption of divalent minerals, particularly calcium (Ola and Oboh, 2000). This in effect makes calcium inaccessible by the body, especially for maintenance of strong bones, teeth, co-factor in enzyme reactions, nerve impulse transmission and blood clothing (Unuofin *et al.,* 2017). However, the oxalate concentration discovered in this study is less than the permissible level of 250mg/100g fresh sample (Oguchi *et al.,* 1996). Consumption of our analyzed sample leaves may not be harmful or toxic especially when the vegetable is heated before usage. Excess alkaloids consumption may result in gastro-intestinal and neurological diseases (Islary *et al.,* 2018).

According to Inuwa *et al.* (2011), high alkaloids concentrations can be harmful especially when they reach the lethal dose of 20mg/100g. The highest quantity recorded in the current study is 10.65±0.30 mg/100g in *B. lanceolaria*. Alkaloids are phytochemicals that are sometimes called antinutrients due to their influence on the nervous system, where they impair electrochemical transmission when taken in excessive amounts (Gemede and Ratta, 2014). The result of the present study is lower than the reported result of Essack *et al.* (2017). Abifarin *et al.* (2020) reported the alkaloid content in *Heteromorpha arborescens* (Spreng.) Cham. & Schltdl. leaves which is comparable with our findings in *Z. oxyphyllum* and *R. serrata*. Njoku *et al.* (2016) reported the alkaloid content in *Synsepalum dulcificum* (Miracle Fruit) Berry (14.17mg/100g) which is higher than our result. Mwakalukwa *et al.* (2016) detected alkaloid content in *Crotalaria laburnoides* Klotzsch leaves from Iramba district, Tanzania which is higher than the present study. A study conducted by Asemave and Ode (2018) reported the antinutritional content in seed, pulp and peel of *Aframomum angustifolium* (Sonn.) K. Schum which is comparable to our findings.

The highest tannin content recorded in *R. serrata* is 12.51±0.13mg/100g. The least tannin content (8.35±0.04mg/100g) is determined from the leaves of *Z. oxyphyllum*. The tannin content of the investigated plants is lower than that of *Colocasia esculenta* (243.06mg/100g) cultivated in Ethiopia (Adane *et al.*, 2013) and *Corchorus oliterius* plant (18.16mg/100g) recorded by Kiliobas *et al.* (2019). The tannin content detected in our samples is comparable to the findings of five indigenous spices used by the Karbi Group of Assam, India as reported by Engtipi and Raju, (2022). Tannin is an antinutritional component found in food that inhibits the enzymes amylase, lipase, trypsin and chymotrypsin from executing their activities efficiently. As a result, the protein's nature is deteriorated, and iron absorption is impeded (Yasir and Ahmad, 2018). Yimer *et al.* (2023) detected the high amount of tannin content in *Solanum nigrum (233.3mg/100g) and Vigna membranacea (175.6 mg/100g)* consumed in South East Ethopia as compared to our results. Kumar and Shiddamallayya (2021) reported the tannin content in some wild edible plants which is higher than the present study. Kiliobas *et al.* (2019) also detected the higher amount of tannin content of *Corchorus oliterius* plant than the present study. Antinutrients in plants can defend themselves and reduce the optimal amount of nutrients like proteins, vitamins and minerals that are exploited. The antinutrient amounts in our samples were within permissible limit for ingestion by humans and animals. Since it has been reported that boiling eliminates or inactivates the majority of antinutritional factors. Therefore, it is recommended to consume the food after cooking, since Seal (2020) stated that the concentrations of anti-nutritional compounds in plant food sources were lowered to a level that is safe for human consumption by boiling and microwaving. A modest consumption of vegetables is also recommended because they are essential in improving human health conditions. It can also be stated that consuming our investigated plant samples may not be dangerous or toxic especially because the vegetable is heated before usage.

Traditional knowledge and historical literature are heavily used in the discovery of new medicinal plants (Buenz *et al.*, 2005). Medicinal plants have emerged as a prominent priority in the hunt for novel medicine due to their efficacy in human clinical trials and the low side effects of pharmaceuticals developed from medicinal herbs. Phytochemicals have sparked an intense research interest because of their perceived health beneficial effects including anticarcinogenic, antiatherogenic, antiulcer, antithrombotic, antidiabetic, antioxidant, anti-inflammatory, immune modulating, antimicrobial, varodialatory and analgesic effects.

Phytoconsitutent Screening:

In the present study, qualitative phytochemical study showed the presence of wide range of phytochemicals in the leaf extracts of all the plant species studied. Among the solvent extracts used, AE proved to be the most suitable solvent for screening of phytochemicals. Next to AE, ME of all the studied plants proved to be a potent solvent for extraction of secondary metabolites. As depicted in tables, the result exhibited that most of the phytochemicals tested like alkaloids, glycosides, phytosterols, phenols, tannins, flavoniods and steroids were present in extracts of the three plant species. The presence of these groups of phytochemicals in plants indicates the possibility of having a wide spectrum of biological and pharmacological activities. However, saponin was absent in all of the HE, CE and ME of the studied plant species. On the contrary, saponin was present in only ZOAE and BLAE. This result is supported by earlier reports of Kebede *et al.* (2021) where presence of saponin was detected in aqueous extracts of *Discopodium penninervium* and *Polysphaeria aethiopica* leaves. In *Z. oxyphyllum,* glycoside was absent in AE which supports the findings of Ramya *et al.* (2017) that showed the absence of glycosides in *S. cumina*, *Termanalia arjuna*, *Naringi crenulata* aqueous leaf extract. BLAE and RSAE alkaloids were not detected. Similar observations were also recorded by Ramya *et al.* (2017) in aqueous extracts of selected plants. Tannin was absent in ZOME. Similar results were also observed by Kumar *et al.* (2020). All extracts of the plant species confirm the presence of fixed oil and fats except aqueous extracts. In this study, a major source of phytochemicals have been screened and the obtained results satisfies the assumption that ethnomedicinal plants are rich in phytochemicals by confirming the presence of secondary metabolites like alkaloids, saponins, flavonoids, tannins etc. which are known to have medicinal property. The presence of these wide ranges of secondary metabolites in the selected plant species justifies their use in traditional medicine as these phytochemicals are known to have immense therapeutic properties. According to Muhammed *et al.* (2023), certain chemical constituents found in plant extract have various therapeutic properties. Batiha *et al.* (2020) also reported that various plant-derived components such as flavonoids and terpenoids have biological functions that promote therapeutic activities such as anti-carcinogenic, anti-mutagenic, anti-inflammatory and antioxidant capabilities. Phytochemicals such as flavonoids, tannins, saponins, alkaloids and terpenoids have a variety of biological effects including antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer and anticancer capabilities mentioned by Starlin *et al.* (2019).

Phenols are the most abundant group of phytochemicals accounting for the majority of antioxidant activity in plants or plant products (Sulaiman and Indira, 2012). They have also been shown to have a wide range of biological activities such as antimutagenicity, anti-carcinogenicity and the ability to regulate gene expression (Marinova *et al.*, 2005). Flavonoids have attracted a great deal of interest due to the potential health benefits they offer. In recent years, several experimental studies have established the biological and pharmacological capabilities of numerous flavonoids including their antibacterial activity, anti-inflammatory, antioxidant and anti-tumor actions all of which are related with free radical-scavenging activity. Flavonoids have also been shown to have hypoglycemic and anti-diabetic properties. Flavonoids contain antioxidant action which protects cells from oxidative damage and lowers the risk of some malignancies (Mohammed *et al.,* 2014). Tannins which are found in all chloroform, methanol and water extracts are astringent, bitter plant polyphenols that bind and precipitate or shrink proteins and are found throughout the plant world. They were historically thought to be antinutritional although they can be used medicinally in antidiarrheal, hemostatic and antihemorrhoidal compounds. Chung *et al.* (1998) also associate them with analgesic and anti-inflammatory properties as well as providing protection against microorganisms. Antidiabetic and antibacterial properties of alkaloids are well recognized as mentioned by Patel and Mishra, (2011); Gawali and Jadhav (2011). Alkaloids found in plants are utilized in medicines to treat headaches and fever. As described by Pietta (2000), they are also attributed with antibacterial and analgesic characteristics. Plants with alkaloids may also have a hypoglycemic effect via the mechanism of insulin-releasing and insulin-mimicking activity and thus improves postprandial hyperglycemia (Usman *et al.*, 2009) and antimicrobial effects due to the action of intercalates into cell wall and DNA of organisms, inhibits release of autacoids and prostaglandins, possess anti-oxidating effects thus reduces nitrate generation which is used for protein synthesis. Furthermore, alkaloid and terpenoids compounds have been shown to have substantial anti-gastric ulcer activity (Abdelhak and Soraya, 2018; Sreeja *et al.*, 2018).

Saponins have been reported to use in traditional medicine formulations as reported by Asl and Hosseinzadeh, (2008). Saponins found in ZOAE and BLAE are generally thought to be antinutrients but they are also thought to be effective in the human diet for cholesterol control. Furthermore, saponins also have expectorant, cardiotonic and hypoglycemic properties as discussed by (Mohammed, 2014). The presence of saponins in both ZOAE and BLAE suggests that this plant can be used for wound healing (Razika *et al.,* 2017). According to the other literature, saponins have an anti-inflammatory action as reported by Just *et al.* (1998). Saponins found in AE interfere with the replication of cell DNA thereby preventing the multiplication of cancer cells (Anila *et al.*, 2000).

Glycosides are recognized to have strong physiological effects with cardiac glycosides being the preferred treatment for congestive heart failure. Furthermore, Boyce and Christy (2004) mentioned that glycosides are known to have laxative, diuretic and antiseptic properties. Oils and fats found in hexane, chloroform and methanol extracts of all plant species has been reported for its gastroprotective, carminative, antiemetic, antibacterial, antifungal, antiviral, antiprotozoal, insect repellents, antioxidant, anticancer, antidiabetic and antimutagenic properties (Reddy, 2019). Phytosterols exert many biological activities as anticarcinogenic effects (Awad and Fink, 2000); immunomodulatory and anti-inflammatory properties (Bouic, 2001; Navarro *et al.,* 2001); antioxidant potential, hypocholesterolemic and antidiabetogenic effects (Marineli *et al.,* 2012; Furlan *et al.,* 2013). The presence of phytosterols in all the four tested solvent extracts put forth a clue that their presence in the samples may be responsible for the use of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* as an anticancer drug by traditional healers. Similarly, steroids detected in extracts of plant species attributes for antibacterial properties (Epand *et al.,* 2007). Presence of saponins, triterpenoids, tannins, flavonoids, cardiac glycosides and steroids revealed that they might be responsible for the obvious anti-inflammatory activities in extracts of the plant samples. This is in alignment with the report of Ahmadiani *et al.* (2000) which stated that flavonoids as well as tannins possess anti-inflammatory effects.

Results of our phytochemical analysis reveals that the plants are rich in compounds like flavonoids, saponins, steroids, alkaloids, tannins, phenols, terpenoids and glycosides which could be responsible for various biological activities. In contrast, solvent extracts prepared by hexane (HE), chloroform (CE), methanol (ME) and water (AE) were used in our investigations to see if the activity and chemical composition of the plants changed when extracted with solvent of different polarity. From the phytochemical screening study, it was testified that AE and ME are ideal and potent solvent systems for extracting various phytochemicals in plants.

Various researchers have examined use of ethnomedicinal plants for the presence of phytochemicals (Philipson, 2001; Edeoga *et al.*, 2005). Preliminary phytochemical studies are significant and helpful in discovering the source of pharmacologically active chemical compounds in plant material leading to their quantitative quantification (Sharanabasappa *et al.,* 2007). According to Samell *et al.* (2018), phytochemicals such as alkaloids, flavonoids, saponins, tannins and others are protective compounds for the treatment of chronic diseases such as cancer, hypertension and diabetes. The existence of these phytoconstituents in various extracts of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* leaves genuinely supports their ethnomedicinal use for the local people in treatment and prevention of various ailments.

Findings of this study show that the detected phytochemicals are bioactive compounds with medicinal properties. These plants are therefore an increasingly useful reservoir of bioactive constituents with significant medicinal value. The results obtained in this study thus suggest that this could be used as a diagnosis tool for standardization and authentication of these plant leaves for medicinal and therapeutic uses in the future.

PHENOLIC AND FLAVONOID CONTENT

The most important plant secondary metabolites with potential health advantages for humans are phenolic and flavonoid. Various extracts of *Z. oxyphyllum*, *B. lanceolaria* and *R. serrata* were measured for the presence of these two main phytochemicals which were detected in qualitative preliminary phytochemical analysis.

It has been claimed that the polarity of the solvent has a substantial effect on the recovery of polyphenols from various sources (Teruel *et al.,* 2015). To extract active compounds with varying polarities, a variety of solvents were used. In the previous investigations, this combination of solvents proved to be quite successful (Stankovic *et al.,* 2010). Results of the current investigation revealed that the highest concentrations of phenolic and flavonoids were found in AE and ME indicating that water and methanol are particularly efficient solvents for extracting phenolic and flavonoids from plant leaves. Furthermore, it was also successfully demonstrated that samples with a high phenolic content contained more flavonoids than other extracts. In addition, water and methanol are both polar solvents indicating that the majority of the substances in our studied samples are polar in nature which is highly soluble in polar solvents. Therefore, an increase in solvent polarity of the various solvent leaf extracts provided a higher yield of phenolic content. Present results were in agreement with those findings reported in literature where the phytochemicals like phenolic and flavonoid content were influenced by extracting solvents.

Previous research by Turkmen *et al.* (2006) and Lapornik *et al.* (2005) showed that the extraction yield of phenolic and flavonoid content is highly dependent on solvent polarity. Numerous studies on the qualitative content of plant extracts found substantial quantities of phenolics in extracts prepared using polar solvents (Canadanovic-Brunet *et al.,* 2008). Higher solubility of phenol in polar solvents results in higher concentrations of phenol in extracts produced by utilizing the appropriate solvent during extraction (Mohsen and Ammar, 2008). Other authors have also inferred that phenol's high solubility in polar solvents results in high concentrations of these compounds in extracts obtained using polar solvents for extraction (Zhou and Yu, 2004). According to Min and Chun-Zhao (2005), the concentration of flavonoids in plant extracts are affected by extraction processes and the polarity of the solvents used for the extraction.

The measures of the colorimetric analysis of quantitative phytochemicals studies are summarized in the above result section (Table: 4.22.). A variation in total phenolic content was observed among the solvent used of each plant species. In the present study, the total phenolic content recorded in ZOAE is higher than ME followed by CE and HE and is in order range of AE> ME> CE> HE. Similar to our results, polar solvent viz., water and methanol have been recognized as effective solvent for extracting phenolics compounds from *Hyoscyamus gallagheri* by Hossain *et al.* (2019). Ozsoy *et al.* (2008) showed the highest content of total phenolic compounds in *Smilax excelsa* leaves was found in water extract and the lowest was found in ethyl acetate. The maximum recovery percentage and extractable total phenolic content was also recorded in water extracts followed by methanol extracts of Mentha species as reported by Laglaoui *et al.* (2014). Similarly, Lee *et al.* (2007) showed that water is the most suitable solvent for extraction of phenolic compounds from *Pleurotus citrinopileatus.* Romero-Diez *et al.* (2018) reported that aqueous extract of plant sample showed better extracting power for phenolic compounds compared with methanol extract. These results are in agreement with our obtained data. Furthermore, *R. serrata* and *B. lanceolaria* showed the highest phenolic content in ME followed by AE, CE and HE. This finding is in agreement with those previous reports of Banerjee and Bonde (2011) which found that *Bridelia retus*a Spreng bark contained relatively higher levels of total phenolics content in methanol extract as compared to others. The total phenolic and flavonoid content in *A. mongolicum* extracted by pure water was also found higher than that of methanol extract by Wang *et al.* (2019). Taroq *et al.* (2018) reported that water extracts showed higher total phenolic and flavonoid content than methanol extracts in *Laurus nobilis* L. leaf. A higher amount of total phenolic and flavonoid was also recorded in aqueous extract in comparison to methanol and chloroform extract of *Senna alata* as mentioned by Pamulaparthi *et al.* (2016).

Jimenez-Sepulveda *et al.* (2009) determined the total phenolic and total flavonoid content in methanol and water extracts of *Justicia spicigera* leaves, stem and flower. He mentioned that the highest total phenolic and total flavonoid content is found in methanolic extracts than water extracts of *Justicia spicigera* in which the value in both extracts are lower than our obtained datas. But the water extracts of *Justicia spicigera* flower and stem showed higher phenolic and flavonoid content than methanolic extracts. Bag *et al.* (2015) determined the flavonoid content in methanolic extract of three Hedychium species of Manipur valley which is much lower than our recorded value. Firoozi *et al.* (2016) recorded the phenolic and flavonoid content which is lower than our findings. The total phenol and flavonoid content in methanolic extract of *Momordica charantia* L. leaves is higher than water extracts reported by Shodehinde *et al.* (2016). Similar results were obtained for higher phenolic and flavonoids content in methanolic extract of *Phlogacanthus thyrsiflorus* in comparison to aqueous extract. Omar and Zain (2018) also mentioned that the total phenolic and flavonoid content in Phyllanthus species is higher in methanolic extracts than water extracts. Stankovic *et al.* (2014) reported that very high concentration of phenolic content is measured in aqueous and methanolic extracts. But the flavonoid content they detected in water extracts (22.18±0.58 mg QE/g dry extract) was lower than our results.

SOLVENT POLARITY

Based on the obtained values of concentrations of phenolic and flavonoid in the examined extracts of all plant species, it can be summarized that higher concentration of these compounds can be extracted using solvents of high polarity. Results of some authors who have comparatively analyzed the concentration of phenolic and flavonoid compounds in various solvent extracts supports the fact that highest concentration of phenolic and flavonoid compounds is found in polar solvents. Our findings also suggest that polar compounds in the plant matrix are easier to extract with a more polar solvent. Solvent of lower polarity enables to obtain the extracts with a higher concentration of bioactive compounds (Bimakr *et al.,* 2011). Therefore, solvent polarity plays a crucial role in this and other extraction studies. As reported by Tiwari *et al.* (2011), solvent nature used will determine the type of chemicals likely extracted from plant materials. During extraction, organic solvents diffuse into the solid material and solubilize the compound with similar polarity. Difference in the polarizability makes the phytochemicals liable to a variety of specific interactions with polar solvents that lead to polarity dependent extraction yield variation which can explain our present observation. Solvent extraction is commonly utilized for antioxidant isolation and all extraction yields. Because of the varied antioxidant potentials of substances with different polarities, the phenolic content and antioxidant activity of the extracts are largely reliant on the solvent (Laglaoui *et al.,* 2014).

ANTIOXIDANT ACTIVITY

According to Gorinstein *et al.* (2007), the strong antioxidant activity of plant extracts is attributable to the presence of phenolic, tannins and flavonoids components all of which are polar chemicals in hydroalcoholic or alcoholic extracts. Furthermore, Guha (2011) found that polar solvent extracts (lower molecular weight alcohols and aqueous) have stronger antioxidant activity than non-polar (e.g., hexane) or low polar (e.g., chloroform) extracts from 56 different types of plants.

According to many research, the other components of the plant species had strong DPPH radical scavenging activity as well as high antioxidant activity. *Calpurnia aurea* stems and leaves showed substantial activity, with the leaves showing higher potential when tested utilizing DPPH and FRAP standard methodologies (Adedapo *et al.,* 2008). But there are also other reports that non polar solvents showed higher antioxidant properties than polar solvent. Behbahani *et al*. (2007) reported that non-polar solvent extract (viz., chloroform and hexane extracts) was found showing higher antioxidant activity in DPPH activity compared to polar extract (ethanol extract) of *Barringtonia racemosa* leaves. Kumar *et al.* (2022) reported that both polar and non polar solvent extracts showed highest antioxidant activity in *Areca catechu* L. nut. In DPPH assay, antioxidant properties of different solvent extracts follows the order as ethyl acetate > toluene > chloroform > n- hexane > methanol. But in H_2O_2 scavenging assay, the order follows as n-hexane > ethyl acetate > chloroform toluene > methanol. Whereas, in FRAP assay as ethyl acetate > toluene > n-hexane> methanol > chloroform. These differences arise from the fact that each method is based on the generation and application of diverse radicals and species that are actively involved in the oxidative process via various mechanisms. As indicated by Rakholiya *et al.* (2011); Khakhalary and Narzari, (2022), the variance could be owing to the complex nature of phytochemicals included in the extracts or the solvent employed for extraction among other things. As a result, many analytical approaches for determining the efficacy of antioxidants found in plants are required.

DPPH radical scavenging activity

The DPPH assay determined the antioxidant ability of plant extracts by converting DPPH free radicals to corresponding hydrazine with a change in colour and decrease in absorbance at 517nm (Rahman *et al.,* 2015). DPPH has been widely utilized as a free radical to evaluate reducing chemicals (Motlhanka *et al.*, 2014) and is a suitable reagent for investigating compounds' free radical scavenging activities (Duan *et al.,* 2006). According to the DPPH assay, all of the extracts in this investigation contained a significant degree of antioxidant activity in a concentration-dependent way. IC_{50} value was calculated to measure the effectiveness of the plant extracts. IC_{50} is the concentration required to result in a 50% antioxidant activity compared with control (Kusmardiyani *et al.*, 2016). The closer the IC_{50} value of the plant extracts to that of the ascorbic acid (standard), the greater the inhibitory effectiveness (Chand *et al.*, 2018).

A smaller IC_{50} means higher antioxidant activity (Chaouche *et al.*, 2014). Antioxidant properties of different solvent extracts follows the order in DPPH radical scavenging assay as aqueous > methanol > chloroform > hexane in *Z. oxyphyllum* and *B. lanceolaria*. In *R. serrata*, the order follows as methanol> aqueous> chloroform> hexane. In this assay, the polar solvent extracts of all the plants samples exhibited the highest antioxidant activity as compared to non-polar solvent extracts used. The standard ascorbic acid displayed IC_{50} value of 112.79±0.02 µg/ml. In comparison to the standard ascorbic acid, the BLAE are showing good antioxidant activity with lowest IC_{50} value of 113.32 ± 0.32 µg/ml which is very close to the standard. Evaluation of antioxidant activity of various solvent extracts was reported by various researchers. Findings of our study are strongly supported by many earlier studies and are correlated to various reported studies. Kma *et al.* (2020) investigate antioxidant capacity of various solvent extracts of *Apium graveolens* L. leaf with DPPH assay reported aqueous extract exhibited the lowest IC_{50} value of 870µg/ml which indicates higher antioxidant activity in comparison to methanol (IC_{50} = 1100µg/ml) and chloroform extracts (IC_{50} = 2400µg/ml) respectively. It is also reported that water appears to be the best extraction solvent for the extraction of various phytochemical compounds from *Apium graveolens* L. Barbouchi *et al.* (2018) reported that the antioxidant activity of *Pistacia lentiscus* leaves varies according to the location of harvest and observed. IC_{50} values ranging from 500 to 900µg/ml and from 570 to 1130 µg/ml were observed for the aqueous and methanolic extracts respectively. Namukobe *et al.* (2021) examined the antioxidant activity of various solvent extracts of some selected ethno medicinal plants that are used for skin infection in Uganda, East Africa showing that polar solvents such as water can extract active compounds with possible free radical scavenging activity as compared to other solvent. Similarly, Anwar *et al.* (2009) mentioned that aqueous extracts was found to be more effective in recovering highest amounts of antioxidant compounds from different parts of medicinal plant extracts through both DPPH and Ferric Reducing Power Assay. Wintola and Afolayan (2011) determined the IC_{50} value of methanol extract, acetone and ethanol extracts of *Aloe ferox* Mill and observed similar values for aqueous extract, the value was 517µg/ml. Islary *et al.* (2017) evaluated the methanol extract of medicinal plant *Antidesma bunius* fruits which showed IC_{50} value of 395.002 µg/ml in DPPH assay and this antioxidant activity was much lower as compared to our study. Dasgupta and De (2007) determined the antioxidant capacity of some leafy vegetables of India with DPPH assay and reported antioxidant activity with IC_{50} values ranging from 5.81 to 1946µg/ml. The results of our study are also comparable to IC_{50} values of 155µg/ml and 204µg/ml observed in the leaves and bark of extracts of *Goniothalamus velutinus* (Iqbal *et al.*, 2015). Satpathy *et al.* (2011) also reported IC_{50} value (590 to 730µg/ml) in methanol extract of edible fruits *Spondias pinnata* which is very less in comparison to our results.

Scavenging effect on DPPH radical was observed to be higher in methanolic leaf extract as compared to the methanolic extract of stem of *Tinospora cordifolia* procured from the local market of Mysore. The leaf and stem extracts of the methanolic solvent of *T. cordifolia* showed greater percentage inhibition with IC_{50} values of 540µg/ml and IC_{50} values of 740µg/ml respectively which was more than the present study (IIaiyaraja and Khanum, 2011). According to Elufioye *et al.* (2016); Terto *et al.* (2020), the methanolic extracts of *Psorospermum febrifugum* exhibited antioxidant activity at 20µg/ml whereas *Plectranthus amboinicus* has shown promising antioxidant activity at 112.39µg/ml.

The antioxidant capacity of various solvent extract of *Cardiospermum halicacabum* (L.) was evaluated by Arunachalam *et al.* (2020) and reported IC_{50} values ranging from 244.34µg/ml to 1141.03µg/ml which are high as compared to our current study. The study also showed that the polar solvent showed the highest antioxidant activity than non polar which also supports our findings. Methanol extract is found to be a potent antioxidant in comparison to hexane extract. Dubey *et al.* (2022) investigated the IC_{50} value of DPPH free radical scavenging activity of *Acmella uliginosa* methanol and hexane extracts which yielded 153.82µg/ml and 204.37µg/ml respectively and these results are comparable with the current study. Similarly, Sobuj *et al.* (2021) reported the highest antioxidant activity in methanol extract (IC_{50} value of 400 ± 0.12 µg/ml) as compared to water extract (IC_{50} value of 3310 µg/ml) of *Padina tetrastromatica* which is in accordance to the findings of our result in *R. serrata.* In methanol and water extracts of *Gracilaria tenuistipitata* seaweeds, IC_{50} value of 2590µg/ml and 5040µg/ml was recorded which indicates very less antioxidant activity in comparison to methanol and water extracts in the analyzed samples by DPPH assay. Manikandan *et al.* (2017) recorded the IC_{50} value of *Psidium guajava* leaf extracts by DPPH radical and recorded 950µg/ml, 640µg/ml and 800µg/ml in water, ethanol and methanol extracts respectively. Similarly, in *Andrographis paniculata*, the best antioxidant activity in terms of DPPH scavenging strength was displayed by methanol extract (IC_{50}=398.31µg/ml) followed by ethanol (IC_{50}=404 µg/ml) and aqueous extract (IC_{50}=483.29µg/ml) as mentioned by Sinha and Raghuwanshi (2021). But our present findings indicated much greater antioxidant capacity with less IC_{50} value compared to the above studies. Navabi *et al.* (2008) assessed antioxidant activity in methanol extract of *Eryngium caucasicum* trautv and *Froripia subpinnata* and reported IC_{50} values of 270µg/ml and 420µg/ml respectively which indicates much lower antioxidant activity as compared to our findings. Sharma and Shrivastava (2022) reported IC_{50} value of 474µg/ml in methanol extracts of *Cleome viscose* leaf which is less antioxidant than in our study. Mohammed *et al.* (2017) reported that the IC_{50} value of the methanolic extract of seeds extracts of *Calycotome villosa* subsp. Intermedia was found to be 200µg/ml in DPPH scavenging assay. Sanjeevkumar *et al.* (2016) investigated the in-vitro antioxidant activity in chloroform extract of *Bryonopsis laciniosa* and reported IC_{50} values of 251.8µg/ml which is much greater than our observed IC_{50} values. It indicates less antioxidant activity as compared to our chloroform extracts. Pourramezan *et al.* (2022) reported that antioxidant activity of aqueous and methanol extracts was very similar but methanol extracts on average possessed more antioxidant properties of Lactobacillus species. Yeboah and Majinda, (2009) reported the chloroform, n-hexane and SFE extracts of root bark of *Osyris lanceolata* behaved in a similar manner, with IC_{50} values greater than 250µg/ml which indicates low antioxidant capacities than our study. An IC_{50} value of 234µg/ml was calculated in hexane extract of *Spondias tuberosa* (Anacardiaceae) leaves by Cordeiro *et al.* (2018) which corresponds to our findings in hexane extract.

H_2O_2 Scavenging assay

In H_2O_2 assay, a concentration dependent scavenging activity was observed in each sample extract. Ascorbic acid was used as standard which showed the IC_{50} value of 120.5±0.43 µg/ml. The highest antioxidant activity was found in BLME with low IC_{50} value (139.51µg/ml). IC_{50} value observed in this plant is in order of ME > AE > CE > HE. The present study supports the finding of various reported data. Hydrogen peroxide (H_2O_2) scavenging activity of *Andrographis paniculata* plant was observed to be high in methanol extract (IC_{50} = 377.074µg/ml) followed by ethanol extract (IC_{50}=379.06 µg/ml) and aqueous extract (IC_{50} = 467.65 µg/ml) by Sinha and Raghuwanshi, (2021). Manikandan *et al.* (2017) reported the IC_{50} value of *Psidium guajava* leaves extracts on H_2O_2 radical and recorded 1050µg/ml, 800µg/ml, 900µg/ml and 750µg/ml IC_{50} values in water, ethanol and methanol extracts respectively. Torre *et al.* (2017) reported IC_{50} value of 328.96µg/ml in flower and 225.34µg/ml in seed of *Caesalpinia pulcherrima* (L.) Swartz methanol extracts which indicates low antioxidant activity as compared to the present study. Nabavi *et al.* (2012) reported IC_{50} value in methanol extract of *Hyssopus angustifolius* flower (177.9±7.8 µg/ml) which indicates low antioxidant activity as compared to our BLME (139.51µg/ml). The present findings indicate much greater antioxidant capacity compared to the above study. Navabi *et al.* (2008) also reported IC_{50} for scavenging of H_2O_2 in the methanol extracts of *Froriepia subpinnata* aerial parts and *Erythronium caucasicum* leaves, which was 810µg/ml and 1300µg/ml respectively. This study shows much lower antioxidant activity than our present findings. Ozen and Kinalioglu (2008) determined the antioxidant activity of various extracts of *Parmelia saxatilis* where it was shown that aqueous and methanol extracts exhibited more effective hydrogen peroxide scavenging activity than standard BHT (Butylated Hydroxytoluene). Swargiary *et al.* (2019) investigated antioxidant activity of some medicinal plants and reported IC_{50} value ranges from 13.7µg/ml to 950.0µg/ml. The result from *R. serrata* are also comparable to the findings of Singh *et al.* (2021) who reported highest antioxidant activity in hexane extracts as compared to chloroform, methanol, water and acetone extracts of *Murraya paniculata.* Hexane extract of *Funtumia Africana* (Benth.) stapf leaves also showed high antioxidant activity as compared to the chloroform extract in H_2O_2 assay (Hamid *et al.,* 2018).

The accumulation of H_2O_2 causes oxidative stress and inflammation, which are linked to pathological disorders such as cancer, diabetes and cardiovascular disease (Lux *et al.,* 2012). This is because H_2O_2 breakdown produces the OH radical, which causes lipid peroxidation and damages cellular components (Kurutas, 2015). Plant antioxidants were found to be promising sources for reducing the harmful effects of hydroxyl free radicals. H_2O_2 which is found in low concentrations in food, bacteria, the human body, water and air can easily breakdown into water and oxygen, resulting in the formation of free hydroxyl radicals. These free radicals can cause DNA and lipid damage (Saeed *et al.,* 2012). Although hydrogen peroxide is not extremely reactive, it can occasionally induce cytotoxicity by producing hydroxyl radicals in the cell (Ebrahimzadeh *et al.,* 2010). H_2O_2 scavenging activity is dependent on the phenolic content of the plant extract, which donates electrons to H_2O_2 and so neutralizes it into water. H_2O_2 was scavenged by extracts in a concentration-dependent manner. The ability of samples to scavenge H_2O_2 may be related to their phenolics and other active components, which can donate electrons to H_2O_2 and hence neutralize it to water (Ebrahimzadeh *et al.,* 2010).

Reducing power assay

Different solvent extracts of investigated samples were also subjected to screening for their possible in vitro antioxidant capacity by reducing power assay. The presence of antioxidants in plant extracts causes the reduction of ferric iron (Fe3+) to ferrous iron (Fe2+) by giving away one electron in the reducing power assay. All of the extracts tested showed good reducing power. The extract's ferric reducing power increases as its concentration increases. When extracts are added to the ferric reducing power assay, the yellow colour of the test solution changes to various shades of green, indicating the reducing power of each extract. The presence of an antioxidant causes the Fe3+/ferricyanide complex to be converted to ferrous/Fe2+ forms. Substances with reduction potential combine with potassium ferricyanide (Fe3+) to generate potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferrous complex, which has a maximum absorption at 700nm. Reducing power is linked to antioxidant activity and may be a good predictor of antioxidant activity (Oktay *et al.,* 2003).

In this study, after data analysis, it was confirmed that all the extracts exhibited some degree of reducing power. It is apparent from the table that the reducing power of all investigated plants was high in polar solvent like methanol and water extracts in comparison to chloroform and hexane extract. In *Z. oxyphyllum*, the ferric reducing power were in the order of AE > ME > CE > HE. Soriana- Santos *et al.* (2016) evaluated the antioxidant activity of aqueous and methanol extract of *Pleurotus ostreatus* and showed that the extracts of dried samples had higher reducing power than the extracts of fresh samples and they also tend to show greater reducing power by aqueous than methanolic extracts. Anwar *et al.* (2009) reported that the aqueous organic solvent extracts of some tested plant materials depicted good reducing power as compared to the respective absolute organic solvents. But in *B. lanceolaria*, the highest antioxidant activity was high in ME as compared to other. These results are in agreement with Nguyen and Eun (2011) who reported that the reducing capacity of the methanol extracts of *Terminalia nigrovenulosa* was higher as compared to other extracts used. This investigation also confirms that polar solvent methanol was the most efficient solvent for extraction of reducing agents from the selected plants which is in agreement with previous findings of Amensour *et al.* (2010) and Arabshahi-Delouee and Urooj, (2007). Dubey, (2022) reported that the reducing power activity of *Acmella uliginosa* methanol extract is found to be a potent reducing agent in comparison to hexane extract. Wintola and Afolayan (2011) reported the highest reducing power in methanol extract as compared to aqueous extract in whole leaf extract of *Aloe ferox* Mill. Moshahid *et al.* (2012) reported that the highest reducing power in methanol extracts than hexane extract of *Cassia fistula* fruit and seed extracts. Ozen and Kinalioglu (2008) determined the antioxidant activity of methanol and aqueous extract of *Parmelia saxatilis* where they showed that methanol extracts exhibited more effective reducing power than aqueous extract. Seal and Chaudhuri, (2015) investigated the antioxidant activity of various solvent extracts of wild leafy vegetables of Meghalaya and reported that the highest reducing power was exhibited by the methanol extract as compared to other extract. It also indicates that polar solvents were important for obtaining fractions with high antioxidant activity and total phenolic content. Ramanibai *et al.* (2019) analyzed the various solvent extract of *Annona reticulata* leaves exhibited and found that chloroform extract has higher reducing power activity as compared to other solvent extracts and this supports the findings in *R. serrata*.

The antioxidant activity observed are in the order range of CE>ME>AE>HE. Yadav and Malpathak (2016) also showed that non polar solvent viz., hexane extract of *Capparis moonii* leaves showed maximum reducing activity amongst the tested plant extracts of varied polarity. The reducing power assay is frequently used to assess the antioxidant's ability to donate an electron since a compound's reducing capacity can be a good predictor of its prospective antioxidant activity (Yildirm *et al.,* 2000). The presence of antioxidants in the extracts decreased the Fe+3/ferricyanide complex to the ferrous form in this assay. According to Jamuna *et al.* (2011), the extracts' reducing capacity may serve as an indicator of possible antioxidant actions by breaking the free radical chain by donating a hydrogen atom. According to several studies, the reducing power of plant extracts is related to their antioxidant activity (Pan *et al.,* 2008). The reducing capacity of antioxidant components is strongly related to their overall phenolic concentration. Many researchers have found that plant extracts with higher levels of total phenolics have higher reducing power (Siddhuraju and Becker, 2003; Sultana *et al.,* 2007).

Many studies are being conducted to investigate the antioxidant properties of various fruits, vegetables and plant extracts in order to reduce the risk of diseases induced by free radicals (Ullah and Hyun, 2020). Antioxidants derived from medicinal, aromatic, spicy and other plants were investigated in order to create natural antioxidant formulations for food, cosmetic and other purposes (Miliauskas *et al.,* 2004). Perez-Jimenez *et al.* (2008) reported increased consumption of natural antioxidant-rich foods is linked to a lower risk of degenerative diseases, including cardiovascular disease and cancer. Numerous studies have linked a high phenolic diet to the protection of cardiovascular, metabolic, viral, and cancer problems due to the pharmacological and biological activities of phenolic compounds (Khalid *et al.,* 2021). Phenolic compounds can neutralize free radicals, singlet oxygen and superoxide radicals by replacing the aromatic ring of the phenolic components with hydroxyl groups (Farahmandfar *et al.,* 2019). Niciforovi *et al.* (2010) discovered that phenolic compounds, when consumed as food had protective benefits on humans.

Flavonoids are a broad family of polyphenolic components synthesized by plants. They are also a type of natural antioxidant capable of scavenging free superoxide radicals, anti-aging, and cancer prevention (Pandey and Kumar, 2013). The significant antioxidant activity in the current study in various solvent extracts of all samples could be attributed to the presence of flavonoids and phenolics which are known to be capable of donating their hydrogen atoms (Bouaziz *et al.,* 2015). According to Liu *et al.* (2009), the antioxidant activity of phenols and flavonoids was directly proportional to their quantity as the principal antioxidant components. This is consistent with the findings of Rice *et al.* (1997), who found that phenolic compounds and flavonoids have anti-oxidative action in biological systems by acting as scavengers of singlet oxygen and free radicals. The current findings were consistent with those described in the literature, where phytochemicals such as phenolic, flavonoids content and antioxidant activity in the plant were altered by extracting solvents. The isolation of these chemicals from various solvent extracts could lead to additional research into the therapeutic qualities of plants.

The findings which are corroborated by data from Nakamura *et al.* (2017) and Cumbane and Munyemana (2017), support the hypothesis that solvent polarity influences the solubility of polyphenolic chemicals and consequently variations in the antioxidant activity. This study found that leaf samples extracted with polar solvents, such as water and methanol have a greater ability to scavenge free radicals, which could be attributed to the fact that water and methanol, being polar solvents, performed better than non-polar solvents for extraction. Although the water and methanol extracts had more scavenging capacities, it was clear that both extracts had some proton-donating ability, which might serve as free radical inhibitors or scavengers, possibly acting as major antioxidants. The current study also indicates that in the future, these plants may be the primary source of drug supplement as an antioxidant, as antioxidant activity has potential role in the treatment of cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol-induced liver disease, ulcerative colitis, ageing and atherosclerosis. Exploiting these pharmacological qualities entails additional exploration of these active substances through the use of extraction, purification, separation, crystallization, characterization and identification procedures.

GC-MS ANALYSIS OF BIOACTIVE COMPOUNDS

GC-MS is one of the most precise methods for identifying various metabolites found in plant extracts (Dinesh- Kumar and Rajakumar, 2018), because some of these chromatographs include preloaded libraries or databases (NIST and WILEY) that allow the possible identity of the metabolites to be determined by comparing the resulting mass spectra to those found as reference in these libraries (Kim *et al.,* 2019; Wei *et al.,* 2014). The type of the bioactive component recovered is largely determined by its solubility in the extraction solvent, the degree of polymerization of the phenols, and their interaction with other plant ingredients (Choi *et al.,* 2002; El Cadi *et al.,* 2020). However, the employment of diverse membrane filters allows recognizing chemical compounds with varying hydrophobicity and molecule sizes. In the present study, GC-MS investigation led to the identification of diverse bioactive compounds in each of the plant extracts. The compounds include mainly fatty acid methyl ester, terpenoids, alcohols and phenolic acids. According to an extensive literature survey conducted as part of this study, it was found that the identified bioactive compounds have various biological activities like antibacterial, antioxidant, anticancer, antidiabetic, antihypersensitive and antiviral. The biological activities of these compounds are an indication of the medicinal potential of these plants. For instance, n-hexadecanoic acid has the highest peak area percentage (29.78%) for bioactive compound detected in HE, CE and ME of *Z. oxyphyllum* among the identified bioactive compounds. This major identified compound n-hexadecanoic acid in all the extract of *Z. oxyphyllum* was also detected in CE of *R. serrata*. Jisha *et al.* (2016) reported the various biological activities of this compound such as an anti-inflammatory, antioxidant activities, pesticidic and nematicide. N-hexadecanoic acid is a fatty acid, possessesing antimicrobial, antioxidant, antiatherosclerotic, antiandrogenic, anticancer and antitumor activities (Nabi *et al.,* 2022). The n-hexadecanoic acid identified in plant extracts of *Benincasa hispida*, *Carissa congesta*, *Allium nigrum*, *Kielmeyera coriacea*, *Cyrtocarpa procera*, *Labisia pumila* and *Rosa indica* has been reported to possess antibacterial activity (Nabi *et al.,* 2022). Also, using the in-silico method, the n-hexadecanoic acid has been used to design inhibitors specific to phospholipase A (2) by comparing with other known inhibitors as anti-inflammatory agents (Nabi *et al.*, 2022).

2-Dodecanone detected in ZOHE, ZOME and RSME are reported for having potent insecticidal, repellent, and antimicrobial activities as mentioned by Wang, (2019). 2-Tridecanone detected in ZOHE strongly modulates the enzymatic activities associated with the scavenging of H_2O_2 at intra- and extracellular levels (Rubilar *et al.,* 2023). 1-hexadecanol, 2-methyl- identified in ZOHE reported for its anticancer, anti-inflammatory, antimicrobial and antioxidant activities as mentioned by Shalaby *et al. (*2021). 2-Pentacosanone detected in ZOHE has no literature of any biological activities yet. Z, z-6, 28-heptatriactontadien-2-one detected in all extracts except ZOME and BLHE possess vasodilatory properties (Mallikadevi *et al.,* 2012).

Sermakkani and Thangapandian (2011) recorded 3,7,11,15-tetramethyl-2-hexadecen-1-ol in the methanol extract of *Cassia italica* leaf. Similar types of compound were identified in ZOHE, RSCE, RSME, BLCE and BLME in this present study. 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol is a diterpene reported for antimicrobial, anti-inflammatory, anticancer and diuretic activities (Rajalakshmi and Mohan, 2016). 3,7,11,15-Tetramethyl-2-hexadecen-1-ol is also recorded to have anti-tuberculosis, insecticidal, anti-inflammatory, antioxidant and antimicrobial activities. George *et al.* (2018) also reported the presence of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol in methanolic extract of *Kalanchoe pinnata* and reported having biological activities like antimicrobial, antioxidant, antifungal and antitumor. 6-Octen-1-ol, 3,7 Dimethyl-, Propanoate detected in ZOHE and RSCE is very minor compounds mainly used in the industries as flavouring agent (Muthuswami and Fathimath, 2020). Pentadecanoic acid was detected only in ZOHE. Cho *et al.* (2020) evaluated the anti-cancer effects of pentadecanoic acid in human breast carcinoma MCF-7/stem-like cells (SC), a cell line with greater mobility, invasiveness and cancer stem cell properties compared to the parental MCF-7 cells. The study showed that pentadecanoic acid exerted selective cytotoxic effects in MCF-7/SC compared to the parental cells. It was also evident from their study that pentadecanoic acid can exert cytotoxic effects in MCF-7/SC and MCF-10A with lesser cytotoxicity to MCF-7/SC. Furthermore, pentadecanoic acid suppressed the stemness of MCF-7/SC through targeting the JAK2/STAT3 signaling pathway. In addition, pentadecanoic acid induced cell cycle arrest and apoptosis in MCF-7/SC. This study proposed the use of pentadecanoic acid as a novel JAK2/STAT3 inhibitor in breast cancer therapy. This result supports the traditional use of this medicinal plant in treating cancer as claimed by the tribes. Phytol which was found in all of our samples is a precursor of synthetic vitamin E and vitamin K and was also reported to be showing cytotoxic activity against breast cancer cell lines (MCF7) as reported by Satyal *et al.* (2012).

Hexanedioic acid, bis (2-ethylhexyl) ester identified in ZOHE is an industrial chemical used in Canada in various products such as cosmetics and so on (www.chemicalsubstances.gc.ca). L-(+)-ascorbic acid 2, 6-dihexadecanoate identified in ZOHE, ZOCE and in RSCE is known for its anticancer, antiviral, anti tumor and anti hypersensitive activity (Singh *et al.*, 2022). From ZOHE tetratriacontane is reported for its antibacterial activity (Garaniya and Bapodra, 2014). 1-Heptatriacotanol, a fatty acid recorded in ZOHE was known to exhibit antioxidant and anticancer activities (Hadi *et al.,* 2016; Junwei *et al.,* 2018). Hentriacontane identified in ZOHE, RSHE and RSAE is known for its antioxidative, protection against UV radiation, anti-tumour activity, anti-inflammatory and anticancer activity by Kim *et al.* (2011); Ramalakshmi and Muthuchelian, (2011). 1, 2-Benzenedicarboxylic acid, diisooctyl ester is a bioactive compound which is also called diisooctyl phthalate and phthalic acid has antioxidant properties (Li *et al.,* 2012). 1, 2-Benzenedicarboxylic acid, diisooctyl ester was detected as major bioactive compound found in RSCE with highest peak area percentage (69.89%). In ZOHE and BLHE 1, 2-benzenedicarboxylic acid, diisooctyl ester was detected. An earlier study suggested that 1,2-benzenedicarboxylic acid and diisooctyl ester isolated and extracted in methanol extract from the unripe fruits of *Nauclea latifolia* exhibited antibacterial properties (Padmashree *et al.,* 2018); their presence was also observed in *Jania rubens*, a red alga (Soad *et al.,* 2016). Another study reported by Syad *et al.* (2013) was on the antioxidant and anticholinesterase activities in the dichloromethane extract of *Sargassum wightii*. Krishnan *et al.* (2014) reported 1, 2- benzene dicarboxylic acid, mono 2- ethylhexyl ester exhibited potential cytotoxic activity against HepG 2 and MCF-7 cancer cell lines. This bioactive compound detected in ZOHE and BLHE.

D-limonene is one of the most common terpenes in nature and has well-established chemopreventive activity against many types of cancer. D-limonene detected in ZOCE and RSME has antiproliferative activity and can induce apoptosis in cancer cells. Ye *et al.* (2020) reported that limonene might be used as a potent anticancer agent against human bladder cancer, inducing significant apoptosis with an increase in the expression of caspase-3. The induction of apoptosis by D-limonene in human colon cancer cells and lung cancer was also reported by Yang *et al.* (2013). Garanyl tiglate detected in ZOCE has been found to have a variety of biochemical and physiological effects. It has been found to reduce inflammation and oxidative stress as well as to reduce the production of pro-inflammatory cytokines. It has also been found to have antimicrobial activity against a variety of bacteria including *Staphyloccus aureus* and *Escherichia coli*.

Additionally, it has been found to reduce the production of nitric oxide which has been linked to a variety of diseases, such as hypertension and cancer. 3-Eicosyne detected in ZOCE and BLCE is well versed for its antimicrobial activity (Ram *et al.*, 2018). Butanoic acid, 3-methyl-,3,7-dimethyl-6-octenyl ester detected in ZOCE, RSCE and BLCE has acidulant and arachidonic acid inhibitor activities. It is also reported to inhibit the production of uric acid and increase the activity of the aromatic amino acid by Suriyavathana *et al.* (2016). Umarani and Nethaji (2021) also reported the antimicrobial activity of Butanoic acid, 3-methyl-,3,7-dimethyl-6-octenyl ester. Methyl 11-methyl-dodecanoate detected in ZOCE, BLHE and BLME is reported for its anti-fungal and antioxidant activities (Nithyadevi and Sivakumar, 2015). 6-Octadecenoic acid, methyl ester identified in ZOCE is reported to possess strong analgesic, anti-inflammatory and antipyretic activity (Jaddoa *et al.*, 2016).

Tanti *et al.* (2019) reported that Dichloroacetic acid, Tridec-2-Ynyl Ester Dichloroacetic acid (DCA) detected in ZOCE are used for cosmetic treatments (such as chemical peels and tattoo removal) and as topical medication for the chemoablation of warts, including genital warts. It can kill normal cells as well. Salts of DCA are used as drugs since they inhibit the enzyme pyruvate dehydrogenase kinase. Early reports of its activity against brain cancer cells led patients to treat themselves with DCA, which is commercially available in non-pharmaceutical grade. Cholestan-3-ol, 2-Methylene- detected in ZOCE can exhibit antimicrobial, anticancer, diuretic, anti-asthma and anti-arthritic activities (Jegadeeswari *et al.*, 2012). Methyl 9,10-Methylene-octadecanoate detected in ZOCE and ZOME is no literature record for any biological activity. Dotriacontane detected in ZOCE are known to possess diverse biological activities such as antioxidant, antimicrobial, antitumor and antiprotozoal activities as well as a chemopreventive value (Gallo and Sarachine, 2009). Soosairaj and Dons (2016) reported dotriacontane having antimicrobial, antioxidant and antispasmodic. Shalaby *et al.* (2021) also reported dotriacontane to exhibit antimicrobial, antifungal, anti-inflammatory and cytotoxic activity. The various biological activities of this compound are an indication of the medicinal potential of *Z. oxyphyllum* leaf. Phenol, 2, 4-bis (1,1-dimethylethyl) identified in ZOME has antibacterial activity (Begum *et al.,* 2016). George *et al.* (2018) also identified phenol, 2, 4-bis (1,1-dimethylethyl) in methanolic extract of *Kalanchoe pinnata* and mentioned about its biological activities like antimicrobial, antioxidant, antifungal and antitumor. Methyl 9-Eicosenoate detected in ZOME is not reported for any biological activities yet. Methyl 8,11,14,17-Eicosatetraenoate identified in ZOME is known to exhibit anti-bacterial, anti-oxidant, anti-fungal and anti-tumor (Somashekar *et al.,* 2023). Butyl 9,12,15-Octadecatrienoate detected in ZOME is reported for its biological activities like anti-inflammatory, hypocholesterolemic, cancer preventive and hepatoprotective (Sudha *et al.,* 2017).

Cholesta-22, 24-diene-5-ol, 4,4-dimethyl identified in ZOME reported for its antibacterial and trypanocidal activity (Tyagia and Agarwal, 2017). 2,2-Dibromocholestanone detected in ZOME of is not reported yet for any biological activities. Ergost-25-Ene-3,5,6,12-Tetrol detected in ZOME is reported to have antibacterial and inhibitory activity against *Pseudomonas syringae* bacteria of 36.3 mm (methanolic extract), 40 mm (n-hexane extract), 30.2 (ethyl acetate extract), 29 mm (chloroform extract) by Akbar *et al.* (2020). 5-methyl-Z-5-Docosene identified in ZOAE is reported for antibacterial, antidiabetic and antitumour activities (Ralte *et al.,* 2022). Dodecane, 1-Fluoro- identified in ZOAE possessr antioxidant and antimicrobial activities (Khan *et al.,* 2016; Shedzad *et al.,* 2018). Dodecanoic acid was reported for its antibacterial, antioxidant and anti-apoptotic activity by Nachiyar *et al.* (2020). Paulsamy *et al.* (2012) informed about its didodecyl phthalate vasodilator, antihypertensive, angiotensin AT2 receptor antagonist, uric acid excretion stimulant and diuretic activities. Neophytadiene is identified for its analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant actions (Raman *et al.,* 2012). Furanodienone identified in ZOAE is reported for its anticancer activity by Ahmed *et al.* (2022). Bano and Deora, (2020) refer tetradecanoic acid for its antioxidant, antimicrobial, lubricant, anticancerous and cosmetics. Triacontane detected in RSHE is cited for its antibacterial, antidiabetic and antitumor activities (Mammen *et al.,* 2010). I-Propyl 11,12-Methylene-Octadecanoate detected in RSHE is reported for its antimicrobial activity (Prarthana and Maruthi, 2017). Phthalic acid, butyl nonyl ester detected in

RSHE is known for antifouling by (Ranganathan, 2014). Butanoic Acid, 3-Methyl-, 3,7-Dimethyl-6-Octenyl Ester is known for its antimicrobial activity (Umarani and Nethaji, 2021). 1,2-Benzenedicarboxylic Acid, Mono(2-Ethylhexyl) identified in RSCE shows cytotoxic activity against HepG 2 and MCF-7 cancer cell (Krishnan *et al.,* 2014). Muhammad *et al.* (2021) reported α-amylase inhibitory and antidiabetic activity activity of hexacosyl acetate identified in ZOAE and RSAE.

Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester was recorded in RSHE. Tyagia and Agarwal (2017) reported antimicrobial activity of this compound. Chloroacetic acid, tetradecyl ester recorded in ZOAE, BLHE and BLME in different quantities and is well known for its antioxidant properties (Shyam and Suresh, 2013). Docosanedioic acid, dimethyl ester a long chain fatty acid and a precursor of phosphocholine derivative, is known for its antifungal activity as revealed from the study of Prakash *et al*. (2019). This compound was detected in RSHE. Nonadecanoic acid, 18-Oxo-, Methyl Ester detected in RSHE has not been cited for any biological activity upto date. 1,2-Benzenedicarboxylic acid, butyl cyclohexyl ester identified in RSHE is accounted for antimicrobial and antifouling activity (Paulsamy *et al.,* 2015). Phthalic acid, butyl dodecyl ester detected in RSHE is testified for antifouling (Ranganathan, 2014). I-Propyl 14-Methyl-Pentadecanoate has no reported biological activities. Arsenous acid, tris (trimethylsilyl) ester identified in RSME is reported to possess antiviral, antithyroid and anticatract activity as reported by Alexander and Rosy, (2022). Octadecanoic acid is a saturated fatty acid found in comparatively high concentrations in some plants. Octadecanoic acid is a primary metabolite present in plants, which forms glycerol esters. It has also been shown that octadecanoic acid has antitumor activity in mouse models and is selectively cytotoxic for MOLT-4 leukemia cancer cells due to its interaction with DNA topoisomerase I and its ability to induce apoptosis (Chujo *et al.,* 2003). 11-Octadecenoic acid, methyl ester identified in ZOME possesses antioxidant, anticancer and antiviral activity (Alam *et al.,* 2021). 13-Docosenoic Acid, methyl ester is a fatty acid ester having anticancer activity (Paudel and Pant, 2017) detected in ZOME. Ethyl 9,12,15 octadecatrienoate identified in ZOME is reported for its antimicrobial activity (Hameed *et al.,* 2015).

Squalene detected in BLHE and RSHE is a triterpene and has several beneficial properties. George *et al.* (2018) reported the presence of squalene in the methanolic extract of *Kalanchoe pinnata* and reported anti inflammatory, anti- atherosclerotic, role in skin aging and pathology. It also possesses anti-cancer properties and reduces blood LDL and cholesterol levels (Lozano-Grande *et al.*, 2018), anticancerous, gastro-preventive, hepatoprotective and pesticidal properties (Ukiva *et al.*, 2002; Ganesh and Mohankumar, 2017), anti-inflammatory, immune-stimulant and lipogenase inhibitor (Yamuna *et al.*, 2017). It has a preventive effect on breast cancer, possesses tumor-protective and cardio-protective properties (Newmark, 1999; Rao *et al.*, 1998). Squalene is reported to have anticancer, antimicrobial, antioxidant, chemo preventive, pesticide, anti- tumor and sunscreen (Jananie *et al.*, 2011). Squalene has antioxidant, antitumor, chemopreventive and hypocholesterolemic properties (Spanova and Drum, 2011). 1-heptacosanol is a long chain alcohol possessing antimicrobial and anti-oxidant properties (Al-Abd *et al.*, 2015). Raman *et al.* (2012) reported its nematicidal, anticancer, antioxidant and antimicrobial activities. 1-heptacosanol was detected in BLHE, BLCE and BLME. However, this bioactive compound was absent in AE. The RSCE also reported 1-heptacosanol. The presence of 1-heptacosanol in *Protea caffra* suggests that the plant might have potent antioxidant, nematocidal and antidiabetic properties (Staden *et al.*, 2020). Methyl 6,9-octadecadienoate detected in RSCE and BLCE isolated from *Millingtonia hortensis* could be the potential molecule for the treatment of new as well as resistance cases of leprosy (Kumar *et al.*, 2014). Berdeaux *et al.* (1998) reported methyl 6, 9-octadecadienoate for its antioxidant activities. This compound was found in RSCE and BLCE. 3-methyl-2-(2-oxopropyl) furan detected in RSME is reported for its antioxidant, antimicrobial, anti inflammatory, antibacterial and antipyretic activity (Nithyadevi and Sivakumar, 2015). 1-nonylcycloheptane detected in RSME is a hydrocarbon reported to show antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* in *Thevetia peruvian* and *Plumeria rubra* flowers and leaves (ElZanaty *et al.*, 2022). Hexanedioic Acid, bis (2-Ethylhexyl) Ester identified in RSAE has anti fungal activity (Xue-Na *et al.*, 2012).

Butanoic acid, 3-Methyl-, 3,7-Dimethyl-6-Octenyl Ester has antimicrobial activity (Umarani and Nethaji, 2021).

Hexadecanoic acid, methyl ester was detected in RSME, BLCE and BLME. This bioactive compound has several bioactive properties like antimicrobial, antioxidant, hemolytic, 5-alpha reductase inhibitor and cancer enzyme inhibitors in pharmaceuticals (Ouyang *et al.*, 2012). Compound hexadecanoic acid, methyl ester were also identified in n-RSHE root evaluated using GC-MS by Tiwari *et al.* (2017).

N-tetracosanol-1, an alcoholic compound recorded in RSME and all extract of *B. lanceolaria* except AE is known for its antioxidant and antibacterial properties (Garaniya and Bapodra, 2014). N-tetracosanol-1 compound isolated from chloroform extract of the *Croton bonplandianum* leaves exhibits antibacterial, nematicidal, anticancer, antioxidant and antimicrobial activity (Kuppuswamy *et al.*, 2013). 9-octadecenoic acid (E)-, methyl ester detected in RSME is reported to have anticancer, antioxidant and antimicrobial activity (Asghar *et al.,* 2011). Trans-13-octadecenoic acid, methyl ester detected in RSME and BLME is a bioactive compound having numerous pharmacological activities like anti-inflammatory, antiandrogenic, anticancerous, dermatitigenic, hypocholesterolemic, anemiagenic and insectifuge properties (Krishnamoorthy *et al.,* 2014). Behenic alcohol identified in HE of *B. lanceolaria* is accounted for its anti-viral activity (Herpes simples virus) by Shettar *et al.* (2017). Cis-1-Chloro-9-Octadecene recorded in BLHE is not reported for any biological activity yet (Singh *et al.,* 2022). Di-N-Octyl Phthalate identified in BLCE has antibacterial and cytostatic activity as mentioned by Boudjelal *et al.* (2011); Amalarasi and Jothi (2019). Boudjelal *et al.* (2011) in his study reported that di-n-octyl phthalate was a compound produced by the genus Actinoalloteichus and had antibacterial activity against *Bacillus subtilis*. Another study conducted by Amalarasi and Jothi (2019) found that di-n-octyl pththalate isolated from *Pachygone ovata* (Poir) possessed a cytostatic activity against breast cancer cell.

Bis (2-ethylhexyl) phthalate identified in BLME is reported for its anti mutagenic, antibacterial and larvicidal activity (Javed *et al.*, 2022). He also mentions that Bis (2-Ethylhexyl) Phthalate isolated from *Paraoctopus vulgaris* exhibit anti mutagenic activity. Bioactive metabolite Bis-(2-ethylhexyl) phthalate (BEHP) isolated from LAB species *Lactiplantibacillus plantarum* BCH-1 exhibited potent antibacterial and larvicidal activity with significant acetylcholinesterase inhibition activity and DNA damage against the larvae of *Culex quinquefasciatus* Say. 2h-Benzocyclohepten-2-One, Decahydro-9a-Methyl-trans detected in both BLCE and BLME is reported for its anti-angiogenic effects and anti-tumor efficacy (Alamery and Algaraawi, 2020). 1-Octacosanol identified in BLCE has insecticidal activity (Zavala-Sanchez, 2020). In BLME 9,12-Octadecadienoic acid (Z,Z)-, methyl ester was found. This compound plays an important role in prostaglandin biosynthesis of cell membranes with several biological functions such as anti-inflammatory, antihistaminic, anti-arthritic and hepatoprotective (Henry *et al.*, 2002; Mensah-Agyei *et al.*, 2020). Methyl 9-Methyltetradecanoate detected in BLCE is reported for antimicrobial activities (Chandrasekaran *et al.*, 2008). Oleyl alcohol identified in BLME has anti-tumor or anti-cancer properties (Orienti *et al.*, 2007). N-pentadecanol is a fatty alcohol found in BLCE which possess antioxidant activity (Geetha *et al.*, 2015). It was also reported by Chatterjee *et al.* (2017) for its antibacterial activity.

Cyclotrisiloxane, hexamethyl, the cyclic, unsaturated (23.002%) bioactive compound identified in RSME, RSAE and BLAE. Abdel-Karim *et al.* (2019) reported this compound as antimicrobial agents, antibacterial activity and antioxidant activity and play a critical role in scavenging of free radicals. 4-methyl-2-4-bis (p-hydroxyphenyl) pent-1-ene in the aqueous extract of *Luffa acutangula* peel extract confirmed and they would help in the formation and stabilization of zinc oxide nanoparticles (Alegria *et al.*, 2018). This study also supports our findings in BLAE. Pentadecanoic acid 14-methyl, methyl ester identified in BLME shows antifungal and antimicrobial activity (Akpuaka *et al.*, 2013). Methyl 9-Cis, 11-Trans-Octadecadienoate identified in BLME is a fatty acid ester reported for antibacterial activity (Lalthanpuii and Lalchhandama, 2019). 1-Decanol, 2-hexyl- identified in RSAE was also reported in methanolic extract of *Solena amplexicaulis* and is known to possess antimicrobial activity

(Krishnamoorthy and Subramaniam, 2014). Antimicrobial activity of 1,1,1,3,5,5,5-Heptamethyltrisiloxane has been mentioned by Mereen and Daiz, (2020). Khan *et al.* (2016) reported trisiloxane, 1,1,1,5,5,5- hexamethyl-3,3- bis [(trimethylsily) oxy] for its antioxidant activity. 1,1-Dichloropentane has antibacterial activity (Krishnamoorthy and Kannabiran, 2020). 2-Propenoic acid, 6- methyl heptyl ester is a polyunsaturated fatty acid ester which is a strong antioxidant and free radical scavenger, thus an ideal nutraceutical component for management of diabetes mellitus (Tulucku *et al.*, 2012) was identified in BLAE. 4-Tert-Octylphenol is no records for any biological activity.

GC-MS analysis showed the presence of phytochemical constituents which contribute various biological activities like antimicrobial, antioxidant, anticancer, hypercholesterolemic, anti-inflammatory etc. The plant extracts were rich in bioactive compounds which explain why traditional healers employ the entire plant to treat a variety of diseases. Additionally, a thorough examination of the numerous substances found and their pharmacological assessment may aid in the development of medications with multiple effects for the treatment of cancer and other disorders. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for their therapeutic roles (Singh *et al.*, 2011). Here we report the presence of important compounds in these plants as identified in GC-MS analysis. Thus, this type of study may be a source of information on nature of active principles present in the medicinal plants for drug development.

FUNCTIONAL GROUP ANALYSIS

The study of plants continues principally for the discovery of novel secondary metabolites which possess many pharmacological properties. Functional group analysis is prerequisite in any phytochemical studies which help in determining the chemical composition of a lead compound. The functional group identification is the key step in the process of determining the chemical constituents and it could be elucidated by FTIR. FTIR is a physicochemical analytical technique providing an idea of the metabolic composition of a tissue at a given time. It measures the vibrations of bonds within chemical functional groups and generates a spectrum that can be regarded as biochemical or metabolic finger print of the sample (Griffiths and Haseth, 1986; Surewicz *et al.*, 1983). In this present study, the FTIR analysis was carried out in *Zanthoxylum oxyphyllum*, *Rotheca serrata* and *Blumea lanceolaria* plant sample. FT-IR analysis of selected medicinal plants showed absorption peak at different values. Different functional groups like alcohol (O-H), alkyne, aliphatic compound (C-H), aldehyde compound (C=O), aliphatic amines (C-N), sulfoxide (S=O), alkyl halide (C-Br) etc were found in crude powder of selected indigenous plants using FTIR in the study.

The absorption peak obtained at 3267 and 3274.42 cm^{-1} in *Z. oxyphyllum* and *R. serrata* indicates the presence of alkyne (C-H). Similar results was also recorded by Shah *et al.* (2019) who investigated the functional group of alkyne in *Thymus linearis* leaf extract by FTIR. The infra-red spectrum shows a frequency range 3650-3000 cm^{-1} in *B. lanceolaria* representing the O-H stretching vibration that confirms the presence of aromatic compound (phenolics and alcohol). The findings support the result of Noviany *et al.* (2023) who reported the presence of O-H functional groups in *Sesbania grandiflora*. Visveshwari *et al.* (2017) observed peak at 3354.08 cm^{-1} in the crude methanol extract of *Ceropegia juncea* indicating the presence of O-H alcohol groups. Medium peaks obtained at 2918.56 and 2850.21 cm^{-1} in *Z. oxyphyllum*, 2918.08 and 2850.04 cm^{-1} in *R. serrata* and 2923.28 and 2852.51 cm^{-1} in *B. lanceolaria* indicates the presence of aliphatic compound (C-H stretching).

Detection of hydroxyl groups is an indication of flavonoids, alcohols and phenolic compounds (Kumar and Oanday, 2013). *B. lanceolaria* is an aromatic plant that is confirmed in present research by the detection of aromatic functional groups. The flavonoids are commonly known as catechins, which are comprised of aromatic rings and hydroxyl groups possessing strong antioxidant activities (Peterson *et al.*, 2005). FTIR analysis revealed the presence of flavonoids due to O-H stretching, terpenes due to C-H groups. Similar results were reported by Dhivya and Kalaichelvi (2017). The peaks recognized at 1601.35 and 1601.07 cm^{-1} in both *Z. oxyphyllum* and *R. serrata* respectively which are assigned to the aldehyde compound (C=C) which supports the results found in *Sesbania* *grandiflora* by Noviany *et al.* (2023). The bands at 497.98 cm^{-1} corresponds to C-I at 500 cm^{-1} reveals the presence of alkyl halide group which is found in *Blumea lanceolaria* leaf. Similar results recorded by Theja and Kuber, (2022) who found the functional groups of alkyl halide at the bands 473.33, 431.29, 404.17cm^{-1} in the aqueous extract of *Ficus sagittifolia*. An absorption band at 506.24cm^{-1} in *Rotheca serrata* IR spectrum exhibited C-Br bond indicates the presence of alkyl halides which corresponds to the result of Kavipriya and Chandran (2018) in *Cassia alata* methanolic extract. Maitera and Chukkol, (2016) also observed C-Br stretching at a peak frequency of 650 cm^{-1} peak in the stem bark of *Faidherbia albida*. The absorption peak obtained at 1260.77 cm^{-1} in *B. lanceolaria* indicating the presence of C-N aromatic amines which supports many previous reports of Indhumathi and Arunprasath (2019); Kamble and Gaikwad (2016). Indhumathi and Arunprasath, (2019) identified the functional group in *C. decumbens* extract by FT-IR and reported that the aromatic amines present in the extract. Kamble and Gaikwad, (2016) reported the functional groups of aromatic amine present in extracts of *Embelia ribes* leaves. Shah *et al.* (2019) confirmed aromatic amine at absorption peak obtained at 1271.17 cm^{-1} in *Thymus linearis* leaf extract.

Vanamane *et al.* (2021) identified S=O sulfoxide at absorption peak 1007.66 cm^{-1} in dried leaf and fruit peel extract of *Capparis divaricata* lam which supports our findings S=O sulfoxide in *R. serrata* leaf at 1008.68 cm^{-1}. The frequency range of 1740 cm^{-1} to 1720 cm^{-1} is represented the C=O stretching vibration, confirmed the presence of aldehyde group at 1731.30 and 1737.31 cm^{-1} peak value in *Z. oxyphyllum* and *B. lanceolaria*. The presence of C-H alkanes group in *B. lanceolaria* at absorption band of 1455.76 cm^{-1} supports the result of Mugendhiran and Sheeja (2020). Chandra (2018) confirmed the presence of C-N stretching at 1240.23 cm^{-1} and reported the presence of aliphatic amines. This result supports the finding of *Z. oxyphyllum* and *R. serrata* at 1243.13 and 1247.38 cm^{-1} respectively. The peaks from 1400-1000 cm^{-1} are denoted C-N stretching vibration for amine group. This group is detected at peak values 1012 cm^{-1} in *Z. oxyphyllum*. There was no peak observed between the region of 2220-2260 cm^{-1} which indicates that no cyanide group is present in all the three plants. This result shows that our analyzed plant does not contain any toxic substances. The FTIR analysis study has confirmed the presence of functional groups which may be important in the synthesis of bioactive phytoconstituents. The functional groups present in test plants belong to secondary plant metabolites as per researchers explanations (Skoog *et al.*, 2007; Paul *et al.*, 2011) which were confirmed by FT-IR spectrophotometry study that predicted the presence of the groups: O-H, C-H, C-N, C=O, C=C, S=O, C-Br. The presence of these functional groups in our study is indicating various medicinal properties of the plants.

Many researchers also analyzed plant parts by of FT-IR spectrum and indicate that medicinal plants having potential to induce insecticidal, nematicidal and antimicrobial activities due to presence of functional groups as well as secondary metabolites (Subramanian *et al.*, 2017; Amorin, 1999; Anis *et al.*, 2000; Venkanna *et al.*, 2013; Radhakrishnan *et al.*, 2015). Alkanes are present in plenty in more or less all biological organisms. It confers ecological and metabolic functions by proving source of carbon and energy. Amines are the important part of amino acids, building blocks of the protein of living beings. So, amino group play important role in both plants and animals. Present result found in agreement with Venkanna *et al.* (2013) who stated that the *Datura stramonium* have antimicrobial properties due to presence of alcohol, alkynes, esters, amines and alkane functional groups. In *Eucalyptus globules* contained alcoholic (O-H) and amide, carbonyl (C=O) and ether (C-O) functional groups were responsible for antimicrobial activity against different fungus and bacteria (Subramanian *et al.*, 2017). FTIR spectral analysis of *Clitoria ternatea* leaves extract was carried out by Lakshmi *et al.* (2015) and reported that the presence of functional groups such as alkanes, aromatic amines, phenols, primary and secondary amines was responsible for the different therapeutic applications.

Functional groups in the plants can be used in different pharmaceutical products such as for anti-cancers, anti-ulcers, jaundice, headache, stomach ache and anti-inflammatory drugs; or as sources of antimicrobial, antioxidant compounds, etc. (Baker, 1982; Skoog *et al.*, 2007; Maobe and Nyarango, 2013). This may also be the reason why traditionally these plants are used by the locals in the treatment of stomach aches, as anti-inflammatory medicine, etc. Analysis of the data clearly shows that our selected study plants have more or less similar functional groups. From this FTIR spectrum, it is easy to determine the constituents of plants extracts and further analysis for its medicinal properties.

ANTICANCER ACTIVITY

Some plant extracts are important source of cytotoxic compounds and have consistently been an interesting field of research. Numerous edible plant-derived compounds have been linked to the chemoprevention and treatment of cancer as reported by various researchers (Sak, 2014). In addition, the usage of potent biologically active compounds of medicinal plants as chemopreventive agents seems to be very promising. Nowadays, it is important to explore new source of natural medicines because the demand for such drugs is increasing. About 60% of anticancer drugs are developed from medicinal herbs and there are still a variety of unexplored species that have anticancer properties which have not been scientifically validated (Kinghorn and Gupta, 2003: Cragg and Newman, 2005). As a result, the use of natural therapy is an alternative to adverse effects of synthetic drugs (Rao *et al.,* 2004).

Cancer mainly affects women in the form of breast and cervical cancer, from 36 different types of cancer (Khan *et al.,* 2019). In present, various treatments i.e., chemotherapy, radiotherapy, surgery and chemically derived drugs are used in treatment of cancer (Greenwell and Rahman, 2015). Therefore, discovery and development studies are still required to find new and effective anticancer drugs (Solowey *et al.,* 2014). MTT assay is extensively applied in the evaluation of cytotoxic drug therapy serving as an analytical tool for chemotherapy (Bahuguna *et al.,* 2017). Preliminary *in vitro* cytotoxic activity of the plant extract was determined using the MTT test. Only live cells can use the NADH enzyme to convert yellow-colored MTT into a purple-colored formazan product. A cell's capacity to convert MTT and produce colour is lost upon death. The cytotoxic effect of methanolic plant extracts on MCF-7 and HeLa cancer cell lines was investigated in this work. In this investigation, it was discovered that the extracts had concentration dependent cytotoxic effects on both cancer cells. With increase in concentration of the plant extract, cell death also increased which is evidence that cytotoxicity of plant extracts increased with increased in concentration. As the concentration of the plant extracts grew, the percentage of cell viability of MCF-7 and HeLa cells were declined. In Figure: 4.33 and Figure: 4.35, it can be seen that the percentage of cells that were viable dropped at the final concentration (100µg/mL) following a 24hr treatment with the extracts. For MCF-7, the percentage of cell viability ranged from 36.84% to 45.22% and for HeLa cells, it was between 32.21% and 44.13%. As per the ISSO: 10993-5 standard, cell viability percentages exceeding 80% are classified as non-cytotoxic, those falling between 80% and 60% as weak, 60% - 40% as moderate and less than 40% as strong cytotoxicity respectively (Ciorita *et al.,* 2021). Treatment of MCF-7 and HeLa cells with plant extracts also caused morphological alterations in the cells. Microscopic observations showed that the extracts treatment on both cells induced apoptotic features. Such morphological features which are sign of cell death were not seen in the untreated cells (control).

Apoptosis is an important preventive strategy against carcinogenesis because it kills genetically faulty cells (Liu *et al.,* 2015). As a result, inducing apoptosis is a highly desired strategy for cancer treatment (Renvoize *et al.,* 1998). The main disadvantage of the MTT assay is that it cannot distinguish between apoptosis and necrosis as the cause of cell growth inhibition. This approach does not identify the hazardous effects of medicines on the body (Carmichael *et al.,* 1988; Phillips *et al.,* 1990). While anticancer medications cause apoptosis in tumour cells, normal cells become necrotic if the treatments are harmful. MTT tests cannot distinguish between these modes of cell death. As a result, the drug's effects may be largely toxic, damaging healthy cells. These medicines have limited clinical applications. As a result, detecting tumour cell apoptosis is more important than determining tumour cell viability. Dual AO/Et-Br fluorescence staining can identify basic morphological alterations in apoptotic cells. Additionally, it distinguishes between normal, early, late apoptotic and necrotic cells. As a result, AO/EB staining can detect apoptosis both qualitatively and quantitatively (Biffl *et al.*, 1996). Apoptosis, a form of cell death is preferred to eradicate cancer cells above other alternative mechanisms because it is a series of regulated cell events that conduct cellular suicide without causing an inflammatory response or harming neighboring cells. It contrasts from necrosis, which induces membrane rupture and triggers an inflammatory response (Kroemer *et al.*, 1998). Most notably, the apoptotic mode of cell death is occasionally changed in cancer cells.

The study of apoptosis opens the door to tumor-specific apoptosis therapy (Lowe and Lin, 2000). Cytotoxic chemicals can cause apoptosis by activating death signaling pathways in susceptible target cells (Fisher, 1994). AO/EB staining is the most reliable approach for detecting apoptosis (Liu *et al.*, 2015). The AO/EB staining method was performed to analyze the morphological and apoptotic mechanisms of the cell revealing a clear distinction between living, necrotic, early and late apoptotic cells with different colour appearance. AO/Et-Br staining demonstrated that all plant extracts had the apoptotic cells in HeLa and MCF-7 cells respectively and also shows a dose dependent induction of apoptosis. Apoptotic cells exhibited morphological changes that are commonly utilized to identify apoptosis (Brady, 2004). Phase contrast microscopy revealed that the control (untreated) cells retained their original shape. In contrast, cancer cells exposed to the plant extracts for 24, 48 and 72hr displayed characteristic apoptotic morphologies such as autophagy and cell rounding due to shrinkage. After 72hrs of incubation, there was an increase in cell membrane permeability, as demonstrated by apoptotic body separation and the presence of reddish orange colour due to AO binding to denatured DNA. Flow cytometry was used to analyze cancer cells for apoptosis and determine whether the cytotoxicity was caused by apoptosis, which is the apoptotic pathway induced by anticancer drugs. Flow cytometric analysis of *B. lanceolaria* showed higher rates of apoptosis in HeLa cells which is in accordance to the results of the AO/Et-Br staining approach. Present study indicates that the methanol extracts of the studied plants are capable of inducing apoptosis in MCF-7 and HeLa cell lines and this is reported for the first time in the present study. Lastly, specific morphological changes related to apoptosis were detected in the cells that had been treated with the effective concentration.

Previous investigations have shown that the methanol extract of *B. lanceolaria* has considerable anticancer activity against the HeLa cell line in a dose-dependent manner (Saikia *et al.,* 2017). The percentage of cell viability of the extracts decreased as the concentration increased. The current findings are consistent with earlier findings of Saikia *et al.* (2017). Prejeena *et al.* (2017) found that *Costus pictus* leaves had anticancer properties against breast cancer cell lines (MCF-7). Samarghandian *et al.* (2014) found that the rhizome of *Alpinia galanga* can trigger apoptosis in MCF-7 cells. Another study on methanol extracts from *Thymus serpyllum* and *Thymus vulgaris* to MCF-7 cells determined the effective concentrations for triggering apoptosis to be 399.407μg/mL and 407μg/mL respectively (Berdowska *et al.,* 2013). Likewise, the ethanol stem bark extract of *Oroxylum indicum* was reported to do the same in HeLa cells (Lalrinzuali *et al*., 2021). Bakhari *et al.* (2020) reported that *Clinacanthus nutans* extracts induce growth inhibition of cervical cancer HeLa cells.

The cytotoxic characteristics of the extract are directly associated with its phytochemicals, which include tannins, triterpenes, saponins, and polyphenolic compounds (Armania *et al.,* 2013). Man *et al.* (2010) claim that saponins' extensive structural diversity enables them to exhibit anti-tumorigenic effects via a range of anticancer pathways. In human cancer cell lines, natural phenolic compounds have been demonstrated to exhibit lethal effects (Lee *et al.,* 2022). These polyphenols have the ability to suppress cell cycle events, induce apoptosis, and modify signalling pathways in order to eliminate cancer cells. These are just a few of the many ways that these polyphenols can fight cancer. Additionally, polyphenols control the actions of enzymes that promote the growth of tumour cells (Kim *et al.,* 2020). It has been shown that flavonoids inhibit the growth of many types of malignant cells (Benavente-Garcia *et al.,* 2008). Antiproliferative properties are demonstrated by flavonoids belonging to the flavone, flavonol, flavanone and isoflavone families in a variety of cancer cell lines, including colon, prostate, leukaemia, liver, stomach, cervix, pancreatic and breast cancer cell lines. These bioactive substances have a well-established potential to induce apoptosis. According to reports, triterpenoids (C30 compounds) show a lot of promise for the treatment and prevention of a wide range of cancers (Bishayee *et al.*, 2011).

The methanolic extract of *Z. oxyphyllum, R. serrata and B. lanceolaria* showed in-vitro cytotoxic potential as well as induced apoptotic cell death in human breast cancer MCF-7 cell and cervical cancer HeLa cell lines. The apoptosis-inducing effect of *Z. oxyphyllum*, *R. serrata* and *B. lanceolaria* on MCF-7 cell and HeLa cell lines may be due to the presence of naturally active phenol and flavonoid compounds. As revealed through GCMS study, the plants possess naturally occurring terpenoids compounds that can induce apoptosis in cancer cells thereby exhibiting greater anticancer potential examined as evident in this study. From these findings, it is inferred that the investigated plants can be use to develop a potential lead compound for therapeutics and cancer therapy.