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Antioxidant Efficacy of Selected Perennials: *Musa* sp., *Cissus* sp. *and Nyctanthes* sp., Prevalent in South India

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ABSTRACT: The present study was aimed to extract and qualitatively analyse the phytochemicals from different plant parts, perform antioxidant assay and anticancer studies on A_{549}.Three perennial plants with unique identities were selected for the present study that includes: organic variety of *Musa acuminate*, 'Red Dacca', salt tolerant variety of *Nyctanthes arbor-tristis* (Night flowering jasmine) and *Cissus quadrungularis* var. *rotundus,* commonly referred as veldt grape. Among various solvents used polar solvents like Ethanol, Water (aqueous) and Acetone were identified as most potent owing to the extraction of most phytochemicals from different plant parts. Stem ethanolic extract of *Cissus* sp., Aqueous extract of flower bracts *of Musa* sp., and Calyx ethanolic extracts of *Nyctanthes* sp., were considered of utmost significance owing to their antioxidant activity and anticancer efficacy against A549 revealed by their respective IC50 values: 125 µg/ml for *Cissus* sp., 63µg/ml. for *Musa* sp. and 70 µg/ml for *Nyctanthes* sp. respectively. Thenceforth the study makes a comprehensive comparison of chosen perennial plants and their parts for bioactive efficacy.

Keywords: *Cissus quadrungularis* var. *rotundus*, A_{549}, *Nyctanthes arbour-tristis, Musa acuminate*, Salt.

INTRODUCTION

Many medicinal plants have therapeutic effects and can be extracted and used in the preparation of drugs, either directly or in combination with other plant extracts, as is common in developing countries. Traditional healers' use of traditional medicines has played a significant role in the health care of millions of people. The National Cancer Institute (NCI) gathered approximately 35,000 plant samples from 20 countries and tested approximately 114,000 extracts for anticancer activity. Over 3000 plant species with antitumor activity have been identified. Natural products or their derivatives thus accounts for approximately 60% of anticancer compounds and 75% of infectious disease drugs (Deep *et al.*, 2021). However, exploitation of perennial plants for natural product derivatisation is a better choice owing to their easy propagation and availability surviving the era of evolution rather than endangered/endemic seasonal varieties. Antioxidant is any substance or nutrient that, when present in low concentrations relative to an oxidizable substrate, significantly delays or inhibits its oxidation. These can also aid in the repair of existing cell damage that would stop the onset and spread of oxidative diseases such as neurovascular, autoimmune, and cardiovascular diseases, cancer, cataracts, immune system decline, and brain dysfunction (Kumar *et al.*, 2014).

Three perennial plants with unique identities were selected for the present study that includes: organic variety of *Musa acuminate*, 'Red Dacca', salt tolerant variety of *Nyctanthes arbor-tristis* (Night flowering jasmine) and *Cissus quadrungularis* var. *rotundus,* commonly referred as veldt grape. Each plant has several properties in their application like: antihelminthic, antileishmanial, antiviral, antifungal, antibilous, hepatoprotective and immunostimulatory properties. Added, unique properties of these plants also forms the rationale of the present study. Some of these include: *Cissus species* that has antiosteoporotic efficacy with bone-healing characteristics, analgesic, anti-inflammatory, and venotonic properties (Sasi & Tamizhiniyan 2018); *Nyctanthes species* that has antispasmodic, cytoprotective, anti-larvicidal and CNS depressive effects (Tripathi *et al.*, 2014) and *Musa species* that is high in pectin and lignin with diuretic, anti-helminthic and hypoglycaemic properties (Vanitha & Vyshnavi 2020).

The present study was aimed to extract the phytochemicals from different parts of these plants and analyse them qualitatively to identify the potent solvent to extract most of the phytochemicals. The potent extracts were then analysed for antioxidant efficacy using DPPH assay. A novel perspective is thus proposed for the identification of antioxidant activity as these chosen perennial varieties - being salt

tolerant/organic lack records for DPPH activity although other related species/varieties/strains had been exploited much for the same in scientific literature.

MATERIALS AND METHODS

Collection of plant samples: Fruit, Flower, Stem, Leaves of organic Red dacca banana variety were obtained from Thiruvananthapuram district, Kerala. The plant samples of *Cissus quadrangularis* var. *rotundus* were collected from the Tamil Nadu State Horticulture Farm, Vichanthangal, Kancheepuram district. The salt tolerant variety of *Nyctanthes arbor-tristis*-NA1 were obtained from Department of biotechnology, Loyola college, Chennai that had been developed with 25mM sodium chloride consistently for 3 months that were able to flower and reproduce effectively.

Preparation of plant extracts: Different plant parts were chosen for extract preparation owing to their usage in home remedies and applications in alternative medicine also different solvents of varying polarity were tested for each of the following to compare their potency in phytochemical extraction.

a) For *Musa acuminate*: The fresh Fruit, Flower, stem, flower bract and leaves of *Musa acuminate* (Red Dacca) (MAR) were selected, washed under running tap water, chopped into fine pieces and shade dried for 15 days. The dried materials were crushed into coarse powder and then stored in 4 ºC until use. 10 g of the dried sample were subjected to extraction separately using 100 ml of distilled water, 80% hexane and 80% acetone for 24 hours at room temperature using a shaker. Each mixture was filtered through Whatman No. 1 filter paper, and the extraction step was repeated three times. The filtrate was then dried in a rotary evaporator at 40 - 50°C. The crude extracts were weighed and refrigerated until analysis (Sasi & Tamizhiniyan 2018).

b) For *Cissus quadrungularis* var. *rotundus*: Leaf and stem parts of the plant were collected, washed thoroughly, cleaned and shade dried, later were finely powdered using a mixer or grinder. 20 grams of thoroughly ground powder were subjected for extraction using 200 mL of solvents - Ethanol and Hexane respectively in separate conical flasks, kept under constant shaking at 200 rpm for 24 hours. After 24 hrs, it was filtered using a Whatman filter paper No. 1 to collect the filtrate that was evaporated in open dish until the final volume was one – fourth of the initial volume. The crude extracts thus obtained were weighed and stored in airtight containers under refrigeration at 4ºC for further assay.

c) For *Nyctanthes arbor-tristis* (NA 1): Fresh leaves and calyx were randomly collected and washed under tap water. The leaves were cut into small pieces and dried in a hot air oven at 65°C for 10 minutes, whereas the calyxes were sun dried for 2 days. The dried leaves were finely powdered using a mixer grinder and used for subsequent extraction process. The leaf powder and dried calyx were extracted with 3 different solvents, namely ethanol, acetone and petroleum ether. 10 g of dried leaf powder and 100 ml of solvent was used for the extraction process. The mixture was taken in an airtight plastic bottle and kept on a rotary shaker for 72 hours. After 72 hours the extract was concentrated on a water bath at 60°C till complete evaporation of the solvent. For the preparation of calyx extract, 2 g of dried calyx was ground in a mortar and pestle using 20 ml of solvent. The extract was centrifuged at 5000 rpm for 20 minutes. The supernatant was used for phytochemical analysis.

The crude extract thus obtained for different extracts from different plant parts were weighed and reconstituted with their respective solvent for a final concentration of 0.1 g/ml.

Qualitative phytochemical analysis: The qualitative phytochemical analysis was carried out using standard procedures on different plant samples for the identification of alkaloids, glycosides, carbohydrates, phenols, tannins, terpenoids, flavonoids, proteins and saponins (Abubakar & Haque 2020; Sharma & Patel 2016).

a) Test for alkaloids. *Dragendorff's test*: 500µl of plant extract was taken in a test tube and few drops of Dragendorff's reagent was added and shaken. Appearance of orangish red precipitate indicates the presence of alkaloids.

b) Test for glycosides. *Legal's test*: To 500µl of plant extract, an equal volume of sodium nitroprusside was added, followed by few drops of 10 % NaOH. The mixture was shaken and observed for the appearance of blood red precipitate which indicates the presence of glycosides.

c) Test for carbohydrates. *Molisch's test*: To 500µl of plant extract, few drops of Molisch reagent was added. This was followed by the addition of few drops of conc. H_2SO_4 along the sides of the test tube. Formation of violet ring at the junction of two layers implies the presence of carbohydrates.

d) Test for phenols. *Ferric chloride test*: To 500µl of plant extract, few drops of 5% $FeCl_3$ was added. A bluish black coloration denotes the presence of phenols.

e) Test for tannins. *Gelatin test*: To 500µl of plant extract, 500µl of 1% gelatin solution containing NaCl was added and shaken. Appearance of white precipitate shows the presence of tannins.

f) Test for terpenoids

Salkowski's test: 500µl of plant extract was mixed with 1 ml of chloroform and few drops of conc. $H_2SO_4$was added, shaken and allowed to stand. An interface with reddish brown coloration signifies the presence of terpenoids.

g) Test for flavonoids. *Alkaline reagent test*: To 500µl of plant extract, few drops of 10% NaOH was added, followed by addition of few drops of dil. HCl. An intense yellow color solution that turnscolorless on addition of dil. HCl indicates the presence of flavonoids.

h) Test for proteins. *Biuret test*: To 500µl of plant extract, 250µl of 4% NaOH was added. This was followed by the addition 250µl of 1% of $CuSO_4$. Appearance of violet color indicates the presence of proteins.

i) Test for saponins. *Froth test*: To 1ml of plant extract, 4ml of distilled water was added and vigorously shaken. Formation of stable persistent froth indicates the presence of saponins.

The extract that showed highest efficacy of most phytochemicals was regarded as potent extract for each plant and they were only subjected for antioxidant activity by DPPH assay.

Antioxidant activity by DPPH assay:

1, 1 Diphenyl 2- picryl Hydrazyl is a stable (in powder form) free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

(DPPH) + (H – A) → DPPH – H + (A)

Antioxidant reacts with DPPH and reduces it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability (Kumar *et al.*, 2014). The free radical scavenging capacity of the potent extracts of each plant were determined according to Hussein *et al*. (2018). 0.1 mM DPPH solution was prepared in 95% methanol. To 1.5 ml of the solvent, 1.5 ml of plant extract was added that was serially diluted by reducing their concentration in half up to five different concentrations (1.25, 2.5, 5, 10, 20 mg/ml). 0.5 ml of DPPH solution was added to all the test tubes. After 30 minutes of incubation in the dark, the absorbance was recorded at 515 nm in a UV/Visible spectrophotometer. The control was prepared with 1.5 ml of solvent and 0.5 ml of DPPH. The DPPH scavenging % was estimated using the following formula:

% scavenging activity = [(absorbance of control – absorbance of test sample)/absorbance of control] × 100.

Anticancer activity onA549 - lung cancer cell line:

The A549cells were placed in 24 well plates (1×10^5 cells per well) and incubated in 5% CO_2 environment at 37°C. Cells (1×10^5/well) were placed in 24-well plates and incubated in 37^0C with 5% CO_2 condition. Once the cells placed in wells reached confluence, the prepared concentrations of extract from 1000µg/ml to 7.8µg/ml were added and kept in incubator for 24 hours. Then the samples were removed from the well, and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl–tetrazolium bromide (MTT) was added to each well (100µl/well) and incubated for 4 hours. Then 1ml of dimethyl sulfoxide (DMSO) was added in all the wells to dissolve the formed formazan crystals. Each sample was placed in the cuvette; using DMSO as the blank the absorbance value at the wavelength of 570 nm was noted using Ultra-violet (UV) Spectrophotometer. The average absorbance values from three observations were taken. The observed values were tabulated, and the concentration required for 50% inhibition (IC50) was determined graphically. The percentage cell viability was calculated by determining the ratio between A570 of treated cells, and A570 of control cells, multiplied by 100. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS AND DISCUSSION

Qualitative analysis of phytochemicals was performed as follows and the results were tabulated (Table 1) In *Musa acuminate* the aqueous extracts were positive for most of the phytochemicals indicating to be a better solvent rather than hexane and acetone. The order of efficacy was Aqueous extract>Hexane>Acetone. The highest amount of phytochemicals was present in Stem. The order of most phytochemicals present in plant parts analysed was Stem>Flower>Banana>leaf.As aqueous extracts were potent, they were used for phytochemical extraction from flower bract that indicated the presence of flavanoids, tannins, phenols, glycosides, proteins, carbohydrate and terpenoids. Saponins and Alkaloids were absent in aqueous flower bracts but present in flower extracts. Hexane and acetone failed to extract significant phytochemicals like alkaloids in flower extract and phenol and protein in stem extract.

Table 1: Phytochemical analysis of organic variety of *Musa acuminate.*

	Hexane				Aqueous					Acetone			
Tests	**Banana**	**Flower**	**Stem**	**Leaf**	**Banana**	**Flower**	**Stem**	**Leaf**	**Flower bract**	**Banana**	**Flower**	**Stem**	**Leaf**
Flavanoids	+++	++	+++	-	++	++	+	+++	+++	-	+	+++	-
Tannins	-	++	++	-	-	+++	+	-	+	-	-	+++	++
Phenols	-	+	-	-	-	++	+	++	+	-	-	-	+
Glycosides	++	++	++	-	+++	-	-	++	++	+	+	+++	+
Saponins	+++	-	+	-	+	+	-	-	-	-	-	-	+
Alkaloids	+	-	-	+	++	++	-	++	-	+	-	++	+
Protein	-	-	-	-	-	-	+	-	+	-	-	-	-
Carbohydrate	+++	+++	+++	-	+++	+++	+	+++	+	-	+++	+++	+++
Terpenoids	+++	-	+++	-	++	+++	+	+++	++	-	+++	+++	+++

In *Cissus* sp. Alkaloids, tannins, flavonoids, glycosides were found to be present in all the extracts of stem and leaf (Table 2). But terpenoids were extracted only in stem ethanolic extracts. Test for phenol and protein failed and need to be tested via alternative methods for reliability. Thus, Ethanolic stem extracts were potent with most phytochemicals among the chosen plant parts. The phytochemicals identified in the leaves of *Nyctanthes arbor-tristis (NA1)* are: alkaloids, glycosides, carbohydrates, phenols, terpenoids,

flavonoids and saponins. The order of efficacy of the solvents used is: Ethanol > Acetone > Petroleum ether. However, the calyx extract had similar results except for flavonoids. The order of efficacy of the solvents for calyx used is: Acetone > Ethanol> Petroleum ether (Table 3).

Table 2: Phytochemical analysis of *Cissus quadrangularis* var. *rotandus.*

Tests	*Cissus Quadrangularis* var. *Rotandus*			
	Stem		Leaf	
	Ethanol	Hexane	Ethanol	Hexane
Flavonoids	+++	+++	+++	+++
Alkaloids	+	+	+	+
Saponins	+	+	-	-
Tannins	+	+	+	+
Phenols	-	-	-	-
Proteins	-	-	-	-
Cardiac Glycosides	+	+	+	+
Terpenoids	+	-	-	-
Carbohydrates	+++	+++	+++	+++

Table 3: Phytochemical analysis of *Nyctanthes arbor-tristis* (NA 1).

	Leaf			Calyx		
Tests	Ethanol	Acetone	Petroleum ether	Ethanol	Acetone	Petroleum ether
Alkaloids	+	+	+	+	+	-
Glycosides	+	+	+	+	+	-
Carbohydrates	+	+	+	+	+	+
Phenols	+	+	-	+	+	-
Tannins	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Flavonoids	+	-	-	-	-	-
Proteins	-	-	-	-	-	-
Saponins	+	-	-	+	+	-

Many of the bioactive compounds found in plant tissues are labile, and they usually alter after extraction. The group of bioactive compounds extracted from the plant material may differ depending on the polarity of the solvent employed for extraction (Cerdá-Bernad *et al.,* 2022). Polar solvents have a notable extraction yield when compared to the other solvents that suggest most of the phytochemicals in these perennials are highly polar and water soluble. Hence polar molecules can be extracted efficiently than non-polar ones. Further, water being a universal solvent, is more efficient as it has a greater polarity, with short chains of a hydroxyl group that can create a hydrogen bond with the solute increasing its capacity to easily elute the polar molecules (Pin *et al.,* 2010).

Antioxidant activity of plant extracts: Among the tested extracts of *Musa acuminata*, Stem extracts exhibited potent antioxidant activity on DPPH, with the least IC50 value calculated for the aqueous extract (0.64mg/mL). The order of efficacy for antioxidant activity on DPPH was Stem>Banana>Flower>Leaf>Flower Bract. The comparison of radical scavenging activity of whole flower and the flower bract were also showed in Fig. 1 that indicates an average of 27% of DPPH activity of the whole flower is contributed by the flower bracts. Significant antioxidants in extracts of *Cissus quadrungularis* var. *rotundus* revealed that stem ethanolic extract of rotundus variety was highly potent with an IC50 value was 0.69 mg/ml (Fig. 2 and 3) compared to leaf extracts. Further DPPH assay in the salt tolerant plants of *Nyctanthes* sp., showed a minimum of 1.25mg/ml of calyx crude extract of the salt tolerant variety with 51.2% RSA and represented in Fig. 4.

Earlier reports on phytochemicals of *Cissus* sp. suggested the presence of diverse compounds like flavanoids, tannins, phytosterols, alkaloids etc. that aids in scavenging DPPH radicals and also help to combat oxidative stress in plants (Safarzadeh *et al.,* 2014). The elicited compounds present in the salt-tolerant flower calyx which better act as antioxidants in scavenging the DPPH is suggestive of terpenoids as the calyx are rich in their derivatives like crocetin, picrocin, crocin and other apocarotenoids, identical to extracts of saffron (Bhuskat *et al.,* 2007; Sujata *et al.,* 1992). Elicited terpenoids acting as better scavengers with enhanced antioxidant activity was suggested by the studies of Dahham *et al.* (2015). Crocetin and its derivatives are known antioxidants as extensively reviewed in the literature studies of Bhuskat *et al.* (2007); Cerdá-Bernad *et al.* (2022); Magesh *et al.* (2006).

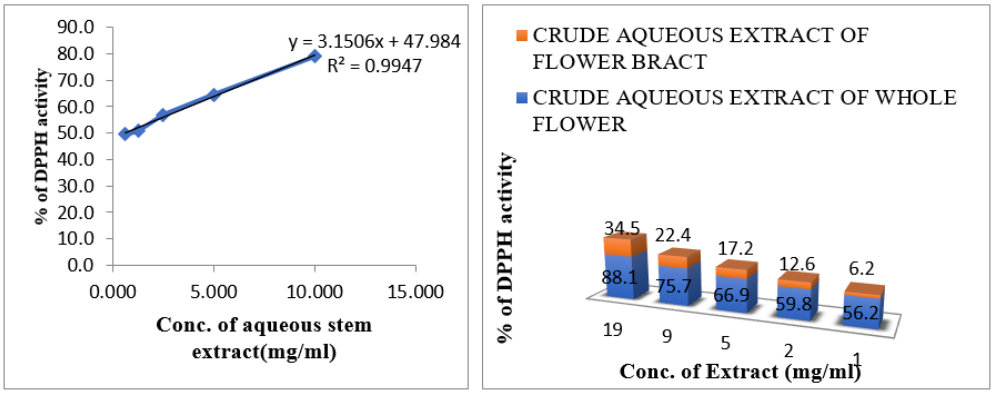

Fig. 1. Antioxidant activity of *Musa acuminate.*

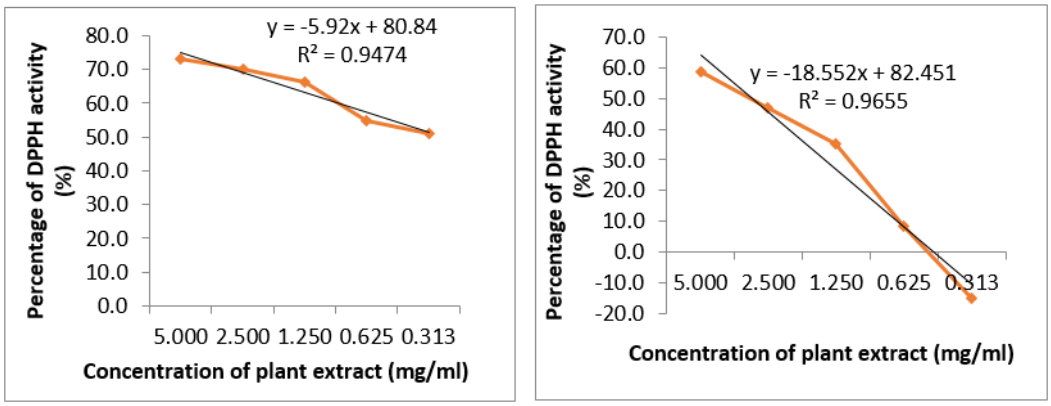

Fig. 2&3. Antioxidant activity of *Cissus quadrungularis* var. *rotundus* - stem and leaf.

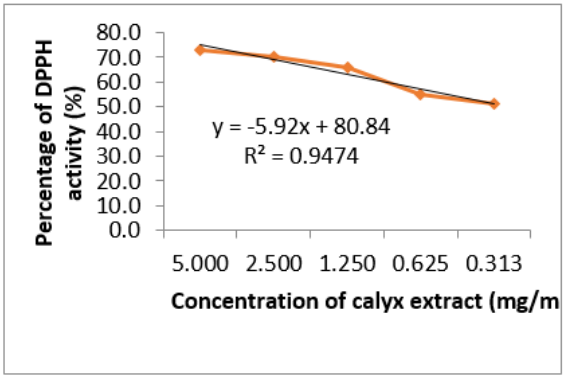

Fig. 4. Antioxidant activity of *Nyctanthes arbor-tristis* NA1.

Anti Cancer Activity. The potent plant extracts with high antioxidant activity that were studied for anticancer activity were: Stem ethanolic extracts of *Cissus* sp. Calyx ethanolic extracts of *Nyctanthes* sp. (NA1) and aqueous Flower bract extracts *of Musa* sp. The results were significantly novel as the effect of these chosen plant extracts on A_{549} lung cancer cell lines were least studied. Findings showed that the extract reduces the A_{549} cell line's viability in a concentration- and time-dependent way. The percentage of cell viability dropped as the fraction's dose and length of treatment with it increased. At 1000 µg/ml, the cells' vitality was insignificant. The percentage of cell viability decreases towards a lower value when the concentration rises from 62.5 to 1000 µg/ml. As the concentration of the extract on A_{549} cells increased, the mortality of cancer cells increased (Fig. 5). The IC50 value for the extract's cytotoxicity activity against the A_{549} cell line were: 125 µg/ml for *Cissus* sp. 63µg/ml. for *Musa* sp. and 70 µg/ml for *Nyctanthes* sp. respectively. Presence of diverse phytochemicals with potent antioxidants has a synergistic effect to confer anticancer efficacy on $A_{549.}$ Vilela *et al.* (2014) revelation on the presence of campesterol, stigmasterol, and beta-sitosterol in appreciable amounts in the fruits of various *Musa* species has shown to cause cell cycle arrest and death in breast and lung cancer cells (Sundarraj *et al.*, 2012). The multiplication of breast, ovarian, and lung cancer cells can also be stopped by phytosterol in accordance with studies done by Canadian researchers (Safarzadeh *et al*., 2014).

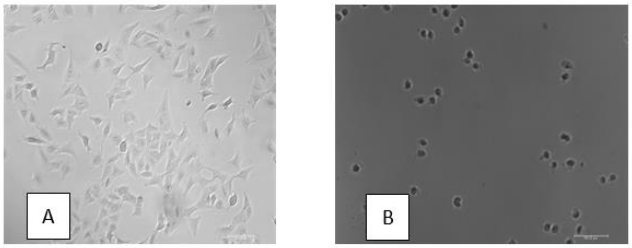

Fig. 5. A and B shows the control – A549 cell line and the treated cell line with plant extract.

Altogether, the study had gained industrial as well as medicinal significance to utilise these plant varieties with potent antioxidant activity, rich in phytochemicals and showing anti-cancer efficacy on lung cancer cell lines. Deep *et al.* (2021) insights and research to exploit these potent phytochemicals can help nature and mankind in future.

CONCLUSIONS

Among various solvents used for phytochemical analysis of chosen plant varieties of *Cissus* sp. *Nyctanthes* sp. and *Musa* sp., polar solvents like Ethanol, Water (aqueous) and Acetone were identified as most potent owing to the extraction of most phytochemicals from different plant parts. Stem ethanolic extract of *Cissus* sp., Aqueous extract of flower bracts *of Musa* sp., and Calyx ethanolic extracts of *Nyctanthes* sp., were considered of utmost significance owing to their antioxidant activity and anticancer efficacy against A549 revealed by their respective IC50 values: 125 µg/ml for *Cissus* sp., 63µg/ml. for *Musa* sp. and 70 µg/ml for *Nyctanthes* sp. respectively. Thenceforth the study makes a comprehensive comparison of chosen perennial plants and their parts for bioactive efficacy.

FUTURE SCOPE

This project proves the bioactive potential of the unexplored medicinal plants like *Cissus quadrungularis* var. *rotundus* and specific varieties of other plants like salt tolerant *Nyctanthes arbor-tristis* NA1 and Organic *Musa acuminate* that can be further tested on infectious diseases that will help many people who are suffering from chronic diseases and immune compromised patients to fight against new diseases. Identification and isolation of specific well -known phytochemicals - like crocetin in NA1, beta sitosterol in *Cissus* sp., Pelarognidin in *Musa* sp. etc. can be exploited in scientific research for bioactivity in comparison with usual variety of these plants that enhance their significance. As well as *in vivo* and *in silico* studies can identify them as potential sources for drug development and docking studies. Propagation of these simple plant varieties and educational awareness can also be a remarkable therapeutic aid for the common people in our day-to-day life.

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Conflict of Interest. None.

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