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Research Article

Evaluation of Bioactive Compounds of Some Common and Traditional Medicinal Plants in Relevance with the Growth of Paddy

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ABSTRACT

India, in terms of its natural resources, is a very rich country since thousands of medicinal plants are found growing and can as well be accessed for their benefits. Manufacturing drugs using plant products has been a field of utmost importance these days. The therapeutic value behind such plants can be exposed to the world only if it is brought out in a consumable form. Assessing the phytochemistry of each plant before determining its medicinal property and the condition that it can target is crucial. Several phytochemical tests and bioassays are therefore necessary to evaluate the various chemical compounds in the plants. In this study, weeds viz., *Leucas aspera* (Lamiaceae), *Tridax procumbens* (Asteraceae), *Justicia adhathoda* (Acanthaceae), *Alternanthera sessilis* (Amaranthaceae), *Phyllanthus niruri* (Euphorbiaceae), *Acalypha indica* (Euphorbiaceae) and six medicinal plants *Rauvolfia tetraphylla* (Apocynaceae), *Achyranthes aspera* (Amaranthaceae), *Tinospora cordifolia* (Menispermaceae), *Bacopa monnieri* (Scrophulariaceae), *Eclipta prostrata* (Asteraceae) and *Clitoria ternatea* (Fabaceae) were chosen to investigate their phytochemical composition, phenolic content, flavonoid content, anti-fungal activity and their effect on paddy seed germination. Extraction was carried out using methanol. The highest phenolic content was observed in extract of *P. niruri* (29.66 mg/g GAE). In contrast *Leucas aspera* showed highest flavonoid content (12.76 mg/g QAE). *P. niruri* at its higher concentration indicated the reduced incidence of fungi like *Alternaria padwickii, Verticillium cinnabarinum* and *Drechslera oryzae* which was from 9% to 2%, 5% to 2%, and 10% to 3%, respectively. These findings indicated the importance of common traditional plants in agriculture apart from their medicinal value.

Keywords: Common traditional plants; Phenolics; Flavonoids; DPPH scavenging; Paddy seeds

INTRODUCTION

Plants produce primary metabolites (Carbohydrates, Lipids and Amino acids) during their early stages of growth and utilize them for further growth and development. Upon reaching a particular phase of growth they start producing secondary metabolites (Alkaloids, Phenolics and Terpenoids) which are essentially of medicinal importance to animals as well as for themselves. The glycosides, alkaloids, saponins, phenolics and other phytochemicals that are present in plants possess healing properties in addition to having no adverse side effects on the human body. To some extent, plant compounds can as well replace chemical-based fertilizers and pesticides that are generally used in agriculture.

Some of such medicinal plants are very rare to be found and some others are very tedious to be cultivated. With increasing urbanization and an elevated trust towards western medicine, some of the important medicinal plants have also turned out to be endangered and at the verge of extinction. Ethno-botany is "The study of the plants which ethnic groups employ for curing human ailments" and evaluating those in use since hundreds of years as home remedies rather than as serious medication such as those from Ayurveda, Siddha and Unani. Most of these plants are common weeds and can be found even today in large numbers and in common places. Hence, in the present study, emphasis has been made to determine the phytochemical content of certain common weeds for their utilization to improve paddy crops, since their availability is more abundant.

MATERIALS AND METHODS

Collection of plant materials

Leaves and tender branches of six well established medicinal plants *Rauvolfia tetraphylla, Achyranthes aspera, Tinospora cordifolia, Bacopa monnieri, Eclipta prostrata* and *Clitoria ternatea* and six common

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weeds used for minor ailments by common traditional people; *Leucas aspera, Tridax procumbens, Justicia adhathoda, Alternanthera sessilis, Phyllanthus niruri* and *Acalypha indica* were collected from a farm area located in Vadagere village of Yelandur Taluk, Chamarajanagara district of Karnataka in India. The aerial parts of the plants thus collected were washed thoroughly in tap water; air dried on blotting sheets for a week and then ground into powder using electric blender.

Preparation of plant extract

The powdered plant material was further subjected to extraction by maceration method. 10 g of powdered material of each plant were taken in 250 ml conical flasks that contained 100 ml methanol and kept for constant shaking for 24 hours in the mechanical rotary shaker. This procedure was repeated twice for each sample by recovering the methanol and replacing the same volume for the remaining plant material within the flask. The methanol extract of each plant was evaporated completely, residue was then scraped and collected in vials and stored at 5°C in the refrigerator for further use.

Phytochemical analysis

Each sample residue of methanol extract was dissolved in 10 ml of distilled water and was subjected to phytochemical evaluation. The analysis was based on qualitative changes in the colour of the reaction mixture. Tests were conducted to confirm the presence of carbohydrates, phenols, proteins, amino acids, saponins, xanthoproteins, terpenoids, alkaloids, flavonoids, quinones and tannins based on the procedures followed by Nethravathi et al. [1].

BIOASSAYS

Estimation of total phenolic content

The total phenolic content was estimated by Folin-Ciocalteu's method. 500 µl aliquots of standard Gallic acid (20, 40, 60, 80, 100 µg) were pipette into test tubes. 0.5 ml Folin-Ciocalteu's reagent was added to each of it and shaken. After 5 minutes, 1.5 ml of 700 mM Sodium carbonate solution was added to every tube and the volume was made up to 5 ml using distilled water. 50 µl of plant extracts at a concentration of 4 mg/ml in methanol were pipetted into different test tubes followed by the addition of the reagents to get a 5 ml reaction mixture. The blank was prepared devoid of plant sample using all the remaining reagents.

The contents were allowed to incubate for 30 minutes at room temperature after which the absorbance at 765 nm was measured using a spectrophotometer (Beckman Coulter- DU730-UV/Vis). A calibration curve was plotted using Gallic acid as the standard. The data for total phenolic contents of the plant extracts were expressed as mg of Gallic acid equivalent weight (GAE) per 100 g of dry mass as described by Kamtekar et al. [2].

Estimation of total flavonoid content

Standard solution of Quercetin at the concentrations of 20, 40, 60, 80 and 100 µg/ml was prepared. The total flavonoid content in each extracted sample was measured following the colorimetric method using a spectrophotometer. 500 µl of each plant extract was mixed with 2 ml of distilled water. 0.15 ml of 5% $NaNO_2$ was added to each tube. 0.15 ml of $AlCl_3$ was added after 5 minutes and 1 ml of 1 N NaOH was added after 6 minutes. The mixture was then made up to 3 ml using 1.2 ml of distilled water. (The flavones and the flavonols, if present in the samples form acid labile complexes using their C-3 and C-5 hydroxyl groups or C4 keto group with Aluminium chloride. Other groups including ortho-dihydroxylgroups of the A or B ring of flavonoids also tend to form complexes with Aluminium chloride). The coloured complexes were spectrometrically measured for their absorbance at 510 nm. following the procedure stated by Kalita et al. [3]. The mixture was shaken thoroughly and the absorbance was read at 510 nm. The results were expressed as mg Quercetin equivalents per 10 g of sample as per the procedure followed by Manukumar et al. [4].

DPPH Free radical scavenging activity

The free radical scavenging activity of the samples was assayed using DPPH free radicals by employing the colorimetric method. 20, 40, 60, 80 and 100 µl of the extracts in methanol were mixed with 480, 460, 440, 420 and 400 µl of 50 mM Tris-HCl buffer (pH 7.4), respectively. 500 µl of the buffer with 1 ml DPPH was used as the experimental control. The change in colour of the solution from purple to yellow upon adding the plant extracts represents the level of antioxidant activity of the compound. More the reduction in colour more is the antioxidant activity of the sample in which the stoichiometric reaction considers the number of hydrogen atoms absorbed by the scavengers. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured by reading the absorbance at 517 nm. Ascorbic acid was used to derive the standard curve and was used to determine the free radical scavenging activity as per the methods followed by Lewis [5].

Blotter test for fungal incidence and seed germination (As per ISTA rules)

The sample that showed the presence of the highest amount of phenolics, flavonoids and the highest percentage of free radical scavenging activity on an average was considered for the evaluation of antifungal activity and germination test.

Seed treatment with organic extracts

400 paddy seeds in four replicates of 100 seeds each of a popular variety 836 around Mysuru, Karnataka, India, were soaked in 10 ml of 1 mg/ml concentration plant extract and such seeds were equidistantly plated on three layers of wet blotters taken in plastic dishes of diameter 9 cm. The seeded plates were incubated for a period of one week under 12/12 h darkness and NUV light at temperature 22 ± 2°C. The incubated seeds in the plates were examined microscopically for the occurrence of seed borne fungi. The percentage incidence of fungi was recorded and the data was tabulated. At the same time, similarly plated seeds were evaluated for the seed germination considering the standard seedling evaluation procedures as described by ISTA rules. In all the cases seeds not treated with plant extract, but soaked in distilled water and plated and incubated in the similar manner were considered as corresponding control for comparison.

RESULTS

The phytochemical analysis of methanol extracts of the plants *Leucas aspera, Tridax procumbens, Phyllanthus niruri, Justicia adhathoda, Acalypha indica, Alternanthera sessilis, Rauvolfia tetraphylla, Tinospora cordifolia, Bacopa monnieri, Clitoria ternatea, Eclipta prostrata* and *Achyranthes aspera* revealed the presence of carbohydrates, amino acids, saponins, xanthoproteins, terpenoids, alkaloids, phenols, flavonoids, quinones and tannins (Table 1). The secondary metabolites of the above plants are active compounds that add to the medicinal properties of the plants.

Table 1: Occurrence of different bioactive compounds in common traditional plants.

Tests	1	2	3	4	5	6	7	8	9	10	11	12	13
L. aspera	-	-	-	-	+	+	+	-	+	-	-	-	+
T. procumbens	-	-	-	-	-	-	+	-	-	-	-	-	-
J. adhathoda	-	+	+	-	+	+	+	+	+	+	-	+	-
A. sessilis	-	+	+	+	+	+	+	+	+	+	-	+	+
P. niruri	+	+	+	-	+	+	+	+	+	+	+	+	-
A. indica	-	-	+	+	-	+	+	-	+	+	-	+	+
R. tetraphylla	-	+	+	+	+	+	+	+	+	+	-	+	-
A. aspera	+	+	+	-	+	+	+	+	+	+	+	+	-
T. cordifolia	-	+	+	-	+	+	+	+	+	+	-	+	+
B. monnieri	+	+	+	+	+	+	+	+	+	+	-	+	+
E. prostrata	+	+	+	-	+	+	+	+	+	+	+	+	-
C. ternatea	-	-	-	-	-	-	+	-	-	-	-	-	-

+= Presence of corresponding bioactive compounds
1=Carbohydrates (Benedict's) ; 2=Phenols ; 3=Amino acids (Ninhydrin) ; 4=Saponin (Foam test) ; 5=Xantho proteins ; 6=Terpenoids ; 7=Alkaloid (Wagner's) ; 8=Phenols (Liebermann's); 9= Flavonoids; 10= Quinones; 11= Protein (Biuret's); 12= Tannins; 13= Flavonoids (Alkaline)

Quantitative analysis

The total phenolic content of the methanol extracts of the plants was determined using the Gallic acid equivalence standard curve where GA in concentrations 20, 40, 60, 80 and 100 µg/mL was taken. The highest phenolic content was recorded in *P. niruri* which is considered to be a common weed used to treat ailments based on tribal methods of healing and the least was observed in *A. sessilis*. It was seen that its phenolic content was higher than the other well-established medicinal plants used in the study (Figure 1).

The total flavonoid content of the methanol extracts of *Leucasaspera, Tridaxprocumbens, Phyllanthus niruri, Justicia adhathoda, Acalypha indica, Alternanthera sessilis, Rauvolfia tetraphylla, Tinospora cordifolia, Bacopa monnieri, Clitoria ternatea, Eclipta prostrata* and *Achyranthes aspera* was determined using the Quercetin equivalence standard curve in which Quercetin was used at concentrations 20, 40, 60, 80 and 100 µg/mL. *L. aspera*, which indeed is a commonly occurring weed with ethno-botanical importance, contained high level of flavonoids. *R. Tetraphylla* showed the next highest amount of flavonoids in it while *J. adhathoda* revealed to contain the least (Figure 2).

The free radical scavenging activity of the plants *L. aspera, T. procumbens, P. niruri, J. adhathoda, A. indica, A. sessilis, R. tetraphylla, T. cordifolia, B. monnieri, C. ternatea, E. prostrata* and *A. aspera* was determined using 2,2-Diphenyl-1-picrylhydrazyl, in which free radicals were scavenged by the sample extracts. At 20 µg/mL concentration, *P. niruri* followed by *L. aspera* and *A. aspera* showed the highest antioxidant activity. The least activity was recorded in *T. procumbens*. At 40 µg/mL concentration of the extracts, *P. niruri* followed by *L. aspera* and *R. tetraphylla* showed the highest antioxidant activity while the least was observed in *A. sessilis*. At 60 µg/mL concentrations, *P. niruri* followed by *A. aspera* and *C. ternatea* showed the highest antioxidant activity whereas the least activity was observed in A. indica. At 80 µg/mL concentration, *C. ternatea* followed by *P. niruri* and *R. tetraphylla* showed the highest antioxidant activity. At 100 µg/mL concentration of the extracts, *C. ternatea* stood first followed by *P. niruri* and *A. aspera* with respect to antioxidant activity whereas *A. sessilis* extract, resulted in the least activity (Figure 3).

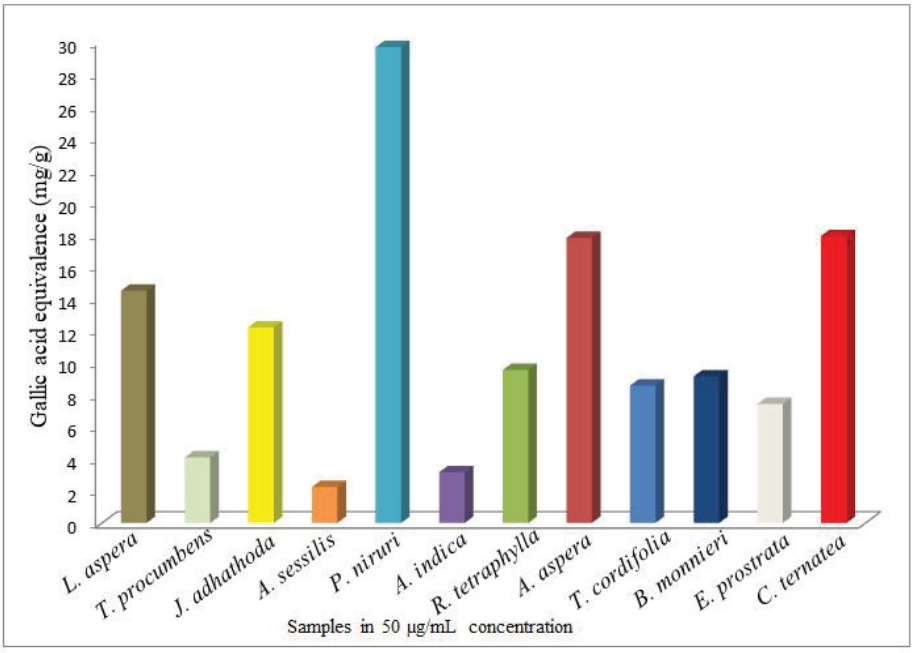

Figure 1: Total phenolic content in different test plants (mg/g GAE).

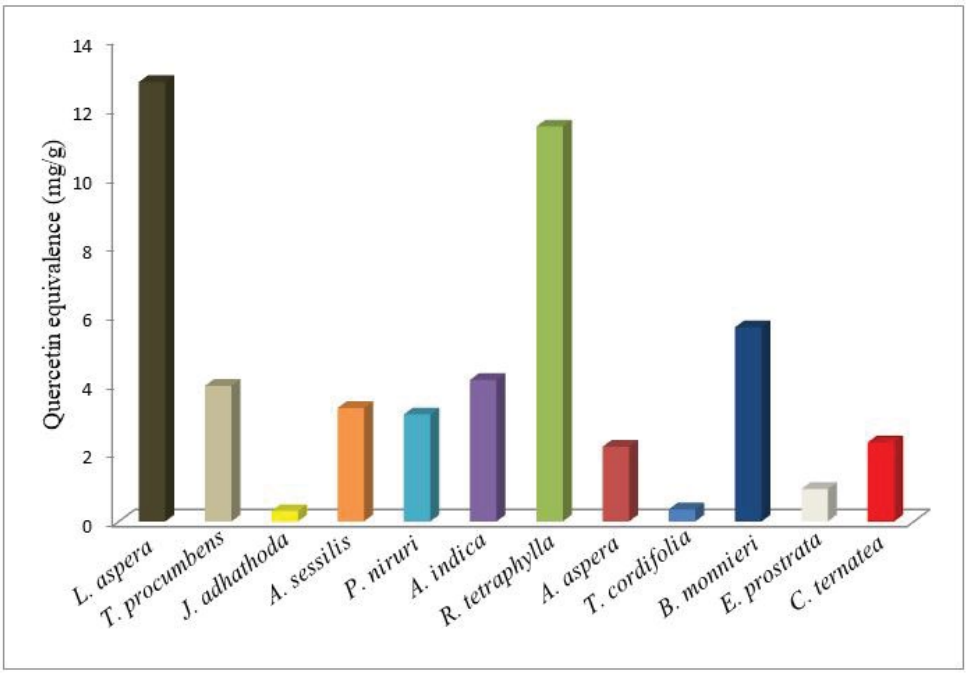

Figure 2: Occurrence of total flavonoid content in different plants (QE).

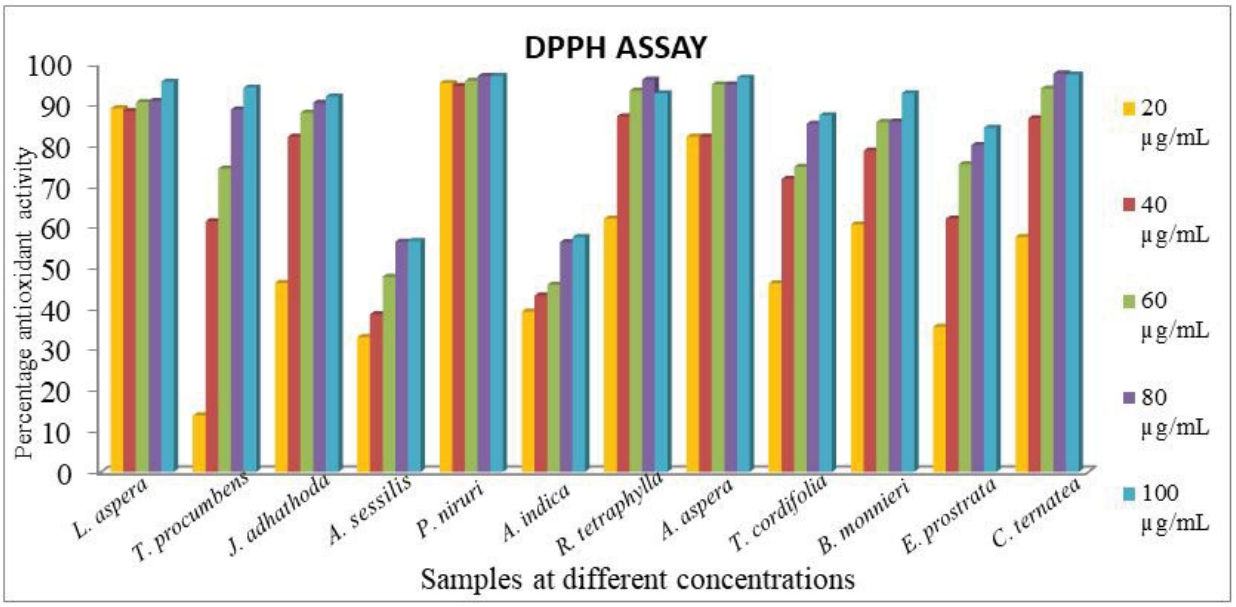

Figure 3: Variable antioxidant activity in the selected plants.

Figure 4: Paddy seeds treated with *P. niruri* showing enhanced germination.

The seeds plated on the Perspex plates were examined after eight days of incubation and the differences in the effect of different plant extracts were recorded. 100% extract of *P. niruri* showed antifungal property, which was confirmed by reduced incidence of *Alternaria padwickii, Verticillium cinnabarinum* and *Drechslera oryzae* compared to the untreated paddy seeds.

The incidence of *A. padwivkii* was 2%, *V. cinnabarinum* was 2% and *D. oryzae* was 3% compared to the incidence of the same on the control being 9%, 5% and 10% respectively. No adverse effect of *P. niruri* was observed with respect to seed germination and seedling growth of paddy (Figure 4).

DISCUSSION

Now-a-days, the use of plant extracts in treating various human, animal and plant diseases is gaining a lot of importance. While some of the well- established medicinal plants that are being employed for the same purpose, since Ayurveda, Siddha and Unani times have retained their properties for ages; some of the less known commonly available weeds which have healing properties are coming into light. The main intention behind looking for healing properties in commonly available weed is to ensure that there exists no difficulty in obtaining resources for preparing medicines. Traditional medicinal plants such as *Rauvolfia serpentina* are scarce in nature and are also very hard to be cultivated. In order to replace the former with a widely grown plant species having similar phyto-chemical properties, testing plants of ethno-botanical importance and those that are available in abundance is crucial.

Treating human as well as plant conditions using plant extracts is also important since synthetic compounds used for the same purpose have proven to be hazardous to the environment and to the human or plant system. Antimicrobial activity of a few such synthetic compounds is vanishing eventually due to the resistance developed within in the pathogens. Plant extracts are organic and are least harmful to the environment and are sure to impose no side effects on humans and plants.

In the present study, the total phenolic, flavonoid content, DPPH assay, antifungal assay and seed germination test results of a set of six common weeds with a set of six well known medicinal plants of ancient medicinal systems were compared. Commonly available weeds showed excellent results for most of the above aspects. All the twelve samples showed high levels of phenolics out of which *P. niruri* contained the maximum. Higher polarity of the solvent is more likely to aid better extraction of plant components. Using Soxhlet apparatus rather than the crude maceration technique could offer better extraction of the metabolites. *Leucas aspera* showed the highest flavonoid content compared to the twelve plants with a value of 12.76 mg/g QE. A study conducted by Ali et al. [6] also is in agreement with the presence of flavonoids in *L. aspera*. The second highest amount of flavonoid was recorded in *R. tetraphylla*, awell established traditional medicinal plant. Review of articles by García et al. [7] also reveals that the flavonoids in food material serve as natural agents against inflammation.

On comparing the percentage inhibition of free radicals of DPPH by each plant, *P. niruri* happened to show 96.93% at 100 µg /mL, the highest of the rest. Ascorbic acid was used as a standard for comparative percentage inhibition. Increasing value of percentage inhibition revealed the chances of having high scavenging activity of free radicals such as reactive oxygen species under *in vitro* conditions.

Studies of Mahdi et al. [8] showed that *P. niruri* had an IC_{50} value of 32.64 µg mass of the extract and had its percentage of inhibition to DPPH 91.57% at a concentration of 200 µg. Studies also claim that the free radical scavenging activity lessens the chances of lipid peroxidation on membranes. The antifungal activity and seed germination stimulating activity of the plant extracts were found varying with respect to varied concentration and metabolites. Antifungal activity and seed germination stimulatory effect of *P. niruri* extract was evaluated by conducting the blotter test. The results revealed that the methanol extract showed a considerably reduced incidence of fungi compared to the control. At the same time, the plant extract did not show any detrimental effect on the germination of the paddy seed. These observations indicated the possibilities of using *P. niruri* extract as a fungicide. 100% plant extract showed reduction of *A. padwickii, V. cinnabarinum* and *D. oryzae* from 9% to 2%, 5% to 2% and 10% to 3%, respectively, by enhancing the germination up to 95% compared to 92% in the control. The plant *P. niruri* is said to have many healing properties. It functions as an anti-hepatotoxic agent, anti-hypersensitive agent, anti-hepatotoxic agent, analgesic, anti- HBV and anti-HIV agent [9]. It is also known to possess the ability to break kidney stones. The hepato protective nature of *P. niruri* also was detected by testing on the liver of rats following a pre-treatment with the extracts of the plant [10]. *P. niruriis* also used as a remedy for Sciatica as well as urinary bleeding [11]. E. prostrata has been used for the treatment of wounds, jaundice, fever, leprosy as well as other skin diseases [12]. Synthetic antioxidants such as Butylated hydroxyl toluene and butylated hydroxyl anisole are effective, yet hazardous to human health. Hence, the importance of finding non-toxic compounds which are natural antioxidants is being unravelled [13]. In a report by Puspita et al. [14]. It is evident that *P. niruri* possessed an activity that prevents the aggregate formation of platelets due to occurrence of a chemical known as Corilagin (B1O galloyl 3,6 (R) hexahydroxy diphenoyl d glucose).

CONCLUSION

All the twelve plants subjected to qualitative analyses showed the presence of carbohydrates, phenols, amino acids, saponins, xanthoproteins, terpenoids, alkaloids, phenols, flavonoids, quinones and tannins. *P. niruri* was found most effective in scavenging free radicals. The idea that the production of organic food products can be simplified and made more effective using plant derived pesticides. Due to the occurrence of bioactive compounds, the plant extracts can be utilized for making useful drugs against several conditions and also as antifungal agents in agriculture. The present work has been carried out with an intention to throw light on the uses of some of the easily available plants and also to emphasize on their ability to act as therapeutic sources in comparison with medicinal plants which are already in the market. Some of the plants which are used by tribes of certain regions in Karnataka since centuries also been assessed for their content.

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