EXPLORATION OF METABOLITES AND THEIR PHARMACOLOGICAL ACTIVITIES OF SELECTED MEDICINAL PLANTS

Subhash L. Kakad[1*],Anant J. Dhembare[1]and Komal G.Kanade[2]
Dept. of Biotechnology[1*,2] and Zoology[1], Padmashri Vikhe Patil, College of Arts, Science and Commerce, Pravaranagar-413 713, Ahmednagar, MS.
(Affiliated to Savitribai Phule Pune University, Pune, MS)

Abstract

This paper deals with the primary and secondary metabolites and their anti-bacterial and anti-fungal activities of common medicinal plants from Sangamner area Maharashtra. The present study was assigned to screen five selected medicinal plants for phytochemical in aqueous and methanol phases during June 2018. Phytochemical such as alkaloids, terpenoids, saponins, phenolic, and flavonoids were screened. The result of the phytochemical analysis showed that all the species were rich in primary and secondary metabolites but *Curcuma amada* and Cissus *quandragularis* reported negativity to some Phenolics, saponins and terpenoids. *Lantana camara L.* showed anti-bacterial activities against all tested bacterial strains. *Cissus quandragularis L,Curcuma amada* Roxb and *Lantana camera L.*showed high anti-fungal activities while *Boerhavia erecta L* and *Phyllanthus niruri L* reported fungal negativity. The phytochemical activities observed in the screened species are indicatives of these plants could be a possible source to obtain new and effective herbal drug to real treatments, hence justified the ethic use of species against various infectious diseases.

Keyword: Metabolites, Medicinal plants, anti-bacteria, anti-fungal, screening.

Introduction

Medicinal plants are the foundation of many important drugs of the modern world. Plants are now playing an important role in many medicines like allopathic medicine, herbal medicine, homoeopathy and aromatherapy. In nature many plants and plants seed provided source of medicine at the earlier times. Plants have proven to be the most useful in curing diseases and provide an important source of pharmacy and medicine. Plants have great significance to the health of individuals.

According to the World Health Origination (WHO) about 4 billion people of the world presently use herbal medicine for their health care or another. Phytochemicals are secondary metabolites and about 12,000 have been isolated but yet to be need of estimation others [1]. Plant products have been part of natural and organic medicines since time immemorial. The metabolites can be derived from any part of the plant like bark, leaves, flowers, seeds, roots etc. which contains active components. The information of the primary and secondary constituents of plants is desirable because such information will be used for the synthesis of complex chemical substances. Such metabolites screening of various plants is reported by many workers [2]. Bioactive compounds in plants are primary and secondary metabolites having pharma-medico benefits to man and animals. These metabolites are of various types and produced within the various sources of plants. The medicinal value of the plants species reflected by chemical substances present in it, which showed definite physiological action in human body. The most important metabolites are alkaloids, cumarins, glycosides, polysaccharides, terpenoids, steroids, flavonoids, tannins and phenolic compounds [3, 4]. Phytochemicals are essential nutrients that are required by human body for sustaining life. Most phytochemicals have anti-oxidant, anti-bacterial anti-fungal etc activities and protect cells against oxidative damage and reduce the risk of developing certain types of cancer. Suggested that phytochemicals, working together with nutrients found in fruits, vegetables, and nuts, may help show the aging process and reduce the risk of many diseases including cancer, heart diseases, and stroke, high blood pressure, osteoporosis and urinary tract infections. This metabolite analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. The medicinal value of these species associated with some chemical substances that produce action on the human body. It is expected that the important metabolite properties recognized in the present study regarding local medicinal plants will be very useful in the curing of various diseases. In the present work, quantitative phytochemical screening and their antibacterial and antiviral activities were carried out on five different local medicinal plants.

Materials and Methods

2.1 **Collection of Materials**: The plant materials were collected from Sangamner ($19^0$34’4 N to $74^0$12’41 E), Ahmednagar District, and Maharashtra during 2016. Thirty different medicinal plants were collected and were used for the purpose of their phytochemical analysis. The plants collected were identified. Fresh and tender leaves of selected plants species were used for phytochemical analysis. Plant species selected during present investigation are shown (Table 1).

2.2 **Preparation of extract**: The leaves of the selected plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were air dried for few days and the leaves were crushed into powder and stored in polythene bags. The plant powder was taken in a test tube and distilled water was added to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of filter paper and filtered extract of the selected plant samples were taken and used for further phytochemical analysis.

2.3 **Phytochemical screening**: Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. **Test for Alkaloids:**

a) Dragendroff's Test: About 0.2 g of the extracts was warned with 2% H_2S0_4 for two minutes. It was filtered and few drops of Dragendroff's reagent were added. Orange red precipitate indicates the presence of alkaloids [5].

b) Mayer's test: To a few ml of filtrate, a few drops of Mayer's reagent were added by the side of the tube. A creamy white precipitate indicates the presence of alkaloids [6].

2. Test for Flavonoid:

a) Alkaline reagent test: Extract was treated with 10 % NaOH solution; formation of intense yellow color indicates presence of flavonoid.

b) NH_4OH test: 3 ml of extract in 10 % NH_4OH solution development of yellow fluorescence indicates a positive test.

c) Mg turning test: Extract were treated with Mg turning and add conc. HCl to this solution add 5 ml of 95 % ethanol, formation of crimson red colour indicates flavonoid.

d) Zn test: A 2 ml extract were treated with Zn dust and concentrated HCl development of red colour indicates presence of flavonoid [7].

3. **Test for Phenolic compounds**: The extract (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds [8].

4. **Test for Saponins**: Two grams of powdered root stem bark and seeds were extracted with 10 ml 70% EtOH by refluxing for 10 min. The filtrate is condensed, enriched with saturated n-BuOH, and thoroughly mixed. The butanol was retained, condensed and used for chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The color and Rf values of these spots were recorded by exposing chromatogram to the iodine vapors [9].

5. **Test for Terpenoids**: An amount of 0.8 g selected plant sample was taken in test tube, and then poured 10 ml of menthol in it, shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml chloroform was mixed in extract of selected plant sample and 3 ml sulphuric acid was added in selected sample extra. Formation of reddish brown colour indicates the presence of terpenoids in the selected plant.

2.4 Antibacterial activities: The antibacterial activity assay was performed for aqueous and methanol extracts and agar well diffusion method for solvent extract [10]. The molten Muller Hinton Agar (Hi Media) was inoculated with the 100 µl of inoculums (1x 10^8 cfu) and poured into the sterilized petri plate. For agar disc diffusion method, the disc (0.7 cm) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plate was incubated over night at 39^0C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain control was maintained in pure solvent were used instead of extract. The result was observed by measuring of zone of inhibition in a diameter. The experiment was repeated three times and means values are reported (Table 4). The obtained results were compared with the standard antibiotics penicillium (100 µg/ disc) and Gentamicin (10 µg/ disc).

2.5 Anti-fungal activities: In order to investigate the anti-fungal activity of the extract, a modified micro dilution technique was used. The fungal spores were washed from the surface of agar plate with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 1.0-107 in a final volume of 100 µl per well. An inoculate was stored at 4^0C for further use. Dilution of inoculate was cultured on solid potato agar to verify the absence of contamination and to check the validity of inoculums. The diameter of the inhibition zones were measured in mm (Table 5).

Results and Discussions

This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as alkaloids, terpenoids, saponins, phenolic, and flavonoids were reported in the samples. The result of the phytochemical analysis showed that the all species screened were rich in primary and secondary metabolites. But *C. amada* and *C. quandragularis* reported negativity to some Phenolics, saponins and terpenoids. The present screened plant species did not showed any phytochemical activity but, negative result do not mean absence of bioactive constituents nor is that plant inactive (Table 2,3).

Terpenoids are present in three plant species. Plant terpenoids are used extensively for their aromatic qualities and play important role in traditional herbal remedies. Sometime terpenoids are added to protein to enhance their attachment to the cell membrane (isoprenylation). Mostly used in perfumes and it is a starting materials for synthesis of vitamin A. Terpenoids are reported to have anti-inflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and anti-bacterial [11]. Flavonoids were reported in all five species tested in the present investigation. Flavonoids are common group of polyphenolic compounds in the human diet and are found in plants. Epidemiologic studies recommend that coronary heart disease is opposed by dietary flavonoids [12]. Alkaloids are present in five plants screened. These are the secondary metabolites found in living organisms. Most alkaloids with biological activity in human affect the nervous system. Plants having alkaloids are used in medicines for reducing headache and fever. These are attributed for antibacterial and analgesic properties [13,14,15].

The antibacterial activities were screed against selected five plants extracts namely *Boerhavia erecta* L,*Ciccus quandragularis L,Curcuma amada Roxb,Lantana camera L,* and *Phyllanthus niruri L* against four bacteria strains namely *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Enterococcus* using the disc diffusion method. All the crude extracts exhibited moderate to good antibacterial activity against the bacterial pathogens tested. Here in and the largest zone of inhibition (25 mm in dm) was recorded against e. coli bacteria to aqueous and methanol extracts of *P.niruri* (Table 4). Minimum inhibitory concentration is the lowest concentration of an anti-microbial that inhibits or kills the visible growth of microorganisms [16]. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. MIC is measured by broth macro dilution method. It appears that crude leaf extract of has antibacterial and antifungal properties and can be used as a novel antimicrobial agent [17].

The antibacterial activity varies species to species because of the antibacterial substances present in plant. There is need to successful evaluation of plant substances and the type of solvent used in the extraction procedures [18]. Researcher mostly preferred water extract, but extracts in organic solvent (methanol) provided more consistent antibacterial activity compared to water. Several workers have identified plant compounds that are known to be antibacterial [19]. Traditional herbal remedies used in world are important sources for discovery of new antibiotics [20]. The antibiotic property of plant compounds that indicates the need for further search in to traditional system but need to reduce possible toxicity present in plant compound.

The antifungal activities of selected five plant extracts namely, *B. erecta C. quandragularis, C. amada, L. camera*, and *P. niruri* were tested against two fungus namely *A. níger* and *T. viridae*. *C. quandragularis, C. amada and L. camara showed high anti-fungal activities while B. erecta and P. niruri reported fungal negativity.* The plants like *C amada* and *C. quandragularis* show the highest zone in water extract (13mm) of inhibition against the *Aspergillus niger* and *Trichoderma viridae* fungus. Antifungal activity of plant extract which might be correlated to the various phytochemical present in their respective extract [21] and also this may be due to the reason that the agrochemicals present in the plants are the supply of natural fungicides, insecticides and pesticides [22,23].

Active compounds may be present in insufficient quantities in the extract to show the activity. Lack of activity can thus only be proved by large dose level and the active compound present in high enough quantities. It is also showed, those gram-positive bacteria are more sensible than gram-negative because due to single layer cell wall in gram-positive bacterium. There are variations in antibacterial activities in species to species due to different phytochemical in species to species. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

Table 1: Showing used plant species.

Sr. No.	Plant species	Family	Vernacular name	Part used
1	*Boerhavia erecta* L	Nyctaginaceae	Punarnava	Leaves
2	*Curcuma amada* Roxb.	Zingiberaceae	Ambihalad	Rhizome
3	*Cissus quandragularis* L.	Vitaceae	Hadjodi	Stem
4	*Lantana camera L.*	Verbenaceae	Ghaneri	Leaves
5	*Phyllanthus niruri* L.	Phyllanthaceae	Bhuiaavla	Leaves

Table 2: Showing primary metabolites of some medicinal plants.

Sr.no.	Name of plants	Carbohydrate	Protein	Lipid
1	*Boerhavia erecta* L.	+	+	+
2	*Curcuma amada* Roxb.	+	+	+
3	*Cissus quandragularis* L.	+	+	+
4	*Lantana camera* L.	+	+	+
5	*Phyllanthus niruri* L.	+	+	+

Table 3: Showing secondary metabolites of some medicinal plants.

Sr. No.	Tests	*B. erecta*	*P. niruri*	*C. amada*	*L. camera*	*C. quandragularis*
1	Alkaloids	+	+	+	+	+
2	Flavonoids	+	+	+	+	+
3	Phenolics	+	+	-	+	-
4	Saponins	+	+	-	+	-
5	Triterpenoids	+	+	-	+	-

+ and - indicates the presence and absence of phytochemical.

Table 4: Plant extract showing antibacterial activities.

Sr. No.	Plant Species	*E. coli*		*K. pnumoniae*		*P. aeruginosa*		*Enterococcus*	
		Water	Methanol	Water	Methanol	Water	Methanol	Water	Methanol
1	*Boerhavia erecta* L.	24	20	13	12	-	-	10	-
2	*Cissus quandragularis* L.	8	7	5	-	12	6	-	15
3	*Curcuma amada* Roxb.	8	-	-	9	15	7	-	15
4	*Lantana camara* L.	9	18	5	6	13	7	15	2
5	*Phyllanthus niruri* L.	25	25	12	15	-	-	8	-

Table 5: Plant extracts showing antifungal activities.

Sr. No.	Plant species	*A. niger*		*T. viridae.*	
		Water	Methanol	Water	Methanol
1	*Boerhavia erecta* L.	-	-	-	-
2	*Cissus quandragularis* L.	11	13	10	12
3	*Curcuma amada* Roxb.	13	12	11	9
4	*Lantana camara* L.	11	10	12	10
5	*Phyllanthus niruri* L.	-	-	-	-

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