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PHYTOCHEMICAL ANALYSIS OF MEDICINAL PLANTS

A.S.Sawalkar and R.M.Kadam
Research student of Department of Botany, Shivaji College Udgir,

Head of the Department of Botany, Mahatma Gandhi College Ahmedpur,

Department of Botany Shivaji College Udgir

Swami Ramanand Teerth Marathwada University Nanded, Maharashtra, India

Abstract
Medicinal plants have bioactive compounds which are used for curing various diseases. In Agriculture, medicinal plants are used as biological control for controlling various diseases of crop plants and increases crop yield. Medicinal plants are very useful for farmers because they have bioactive compounds which show antifungal and antibacterial activities against plant pathogens. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids. Secondary constituents contain terpenoids and alkaloids. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The present study involves fifteen different medicinal plants locally available in Osmanabad and Beed district of Maharashtra. The plant part which is used for the study of the selected medicinal plants were washed, air dried and then powdered. The aqueous extract of those samples were used for the phytochemical analysis to find out the phytochemical constituents in the plants. The main objective of the research work was to check the presence or absence of the phytochemical constituents in all the selected medicinal plants. The results of the phytochemical analysis of these medicinal plants showed that the terpenoids, reducing sugar, flavonoids and alkaloids were found to be present in mentioned medicinal plants. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of new drugs for curing various diseases. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants of Osmanabad and Beed will be very useful in antifungal, antibacterial activities against plant pathogens for controlling various diseases of crop plants.

Keywords: Antibacterial, Antifungal, Anti-inflammation activities, Medicinal plants; phytochemicals.

Introduction

Medicinal plants have bioactive compounds which are used for curing various diseases. In Agriculture, medicinal plants are used as biological control for controlling various diseases of crop plants and increases crop yield. Medicinal plants are very useful for farmers because they have bioactive compounds which show antifungal and antibacterial activities against plant pathogens. Phytochemicals naturally occur in medicinal plants, leaves, vegetables and roots that have defense mechanisms and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds [1]. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas [6]. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro [7]. Plant products have been part of phytomedicines since time immemorial.

This can be derived from barks, leaves, flowers, roots, fruits, seeds [9]. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances [9], [10], [11].

Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects. The plants which have been selected for medicinal use over thousands of years constitute the most obvious choice of examining the current search for therapeutically effective new drugs such as anticancer drugs [1]. Antimicrobial drugs [2], antihepatotoxic compounds.

Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, antiviral and anti-bacterial activities [3]. Terpenoids are very important in attracting useful mites and consume the herbivorous insects [4]. Alkaloids are used as anesthetic agents and are found in medicinal plants [5].

Medicinal plants used for phytochemical analysis are –

1 .*Aegle marmelos:*

Family: Rutaceae

It has been used for centuries as an herbal medicine. It is commonly known as Bael, is indigenous to India and is one of the most useful medicinal plants in India. Its stem, bark, root, leaves and fruits have medicinal value. Other actions like antifungal, antibacterial, antiprotozoal, hypoglycemic, antioxidant, antiviral and cardioprotective effects have been studied using various parts of the plant. Besides its antioxidant properties, *Aegle marmelos* unripe fruit aqueous extract interacts by various other mechanisms in a complex way to elicit its therapeutic effects.

2. Aloe (*Aloe vera*)

Family: Liliaceae

It is the oldest medicinal plant and the most applied medicinal plant worldwide. It has numerous monosaccharides and polysaccharides; vitamins B1, B2, B6, and C; niacinamide and choline, several inorganic ingredients, enzymes (acid and alkaline phosphatase, amylase, lactate dehydrogenase, lipase) and organic compounds (aloin, barbaloin, and emodin). *Aloe vera* helps to soothe skin injuries affected by burning, skin irritations, cuts and insect bites, and its bactericidal properties relieve itching and skin swellings, wrinkles and actively repair the damaged skin cells that cause the visible signs of aging. Aloe is a powerful detoxifier, antiseptic and tonic for the nervous system, immune-boosting and anti-viral properties.

3 .*Eclipta alba* (L.)

Family: Asteraceae

It is commonly known as bhringraj as well as false daisy. It is a weed which grows in tropical and subtropical regions all over the world. It is widely dispersed throughout India, Brazil, Thailand, and China. The herb *Eclipta alba* contains many bioactive components such as coumestans i.e. wedelolactone and demethylwedelolactone, triterpenes, flavonoids, steroids, polypeptides, polyacetylenes and thiophene-derivatives. The plant is commonly used in hair oil all over India for healthy black and long hair, catarrhal jaundice and for skin diseases. *Eclipta alba* constituents of wedelolactone were investigated in the myotoxicity of snake venoms. The herb has been known for its curative properties and utilized as antimytotoxic, analgesic, antibacterial, antihepatotoxic t6, antihemorrhagic, antihyperglycemic, antioxidant, immunomodulatory properties and it is considered as a good rejuvenator too.

4. *Punica granatum*

Family: Lythraceae

Pomegranate is the common name of the ***Punica granatum*** (PG) and belongs to the family Lythraceae. It has much medical significance and has been used as medicine for centuries [16]. The recent studies have investigated that pomegranates are used for the treatment of a number of diseases e.g., diabetes, dysentery, diarrhea, cough, asthma, bleeding disorders, bronchitis, fever, AIDS, inflammation, ulcers, malaria, prostate cancer, hypertension, atherosclerosis, hyperlipidemia, male infertility, infant brain ischemia and obesity.

5. *Psoralea corylifolia*
Family: Leguminosae
Subfamily: Papilionaceae
It is an annual herb growing throughout the plains of India. The plant immense biological importance, and it has been widely exploited since ages for its magical effect against several skin diseases, such as psoriasis, leucoderma and leprosy. ***P. corylifolia*** extracts have found to possess antitumor, antihyperglycemic, antidepressant and antioxidant activities. Its water extract possesses antibacterial properties. Seeds and extract powder are used as diuretic, anthelmintic, laxative and for healing wounds. The major components psoralean and isopsoralean have antitumor, antibacterial and antiviral properties [36].

6. *Bauhinia variegata*
Family: Caesalpiniaceae
Botanical Origin (Kirtikar and Basu, 1994) ***Bauhinia variegata*** Linn. *Bauhinia variegata* Linn (Mountain Ebony) is a medium-sized, deciduous tree, found all through India, climbing to a height of 1,300 m in the Himalayas. The plant is generally utilized by the tribals all through India and mainstream in different indigenous frameworks of drugs like Ayurveda, *Bauhinia variegata* Linn. are hostile to diabetic, against ulcer, hostile to oxidant, nephroprotective, hostile to growth, hepatoprotective, calming, and immunomodulatory, hostile to microbial, hostile to bacterial.

7. ***Tinospora cordifolia***
Family: Menispermaceae
Gulvel is a medicinal plant with various properties of importance for our health. It is a deciduous shrub commonly called "Guduchi" in Sanskrit. Gulvel has several beneficial properties. It may have effective properties on immunity (immunomodulatory), anti-toxin, antidiabetic, antioxidant, antiarthritic, anticancer, antimicrobial activity, and anti-inflammatory properties.

8. *Moringa oleifera*
Family: Moringaceae
Drumstick tree has numerous traditional medicinal uses in many parts of its native and the tree continues to have an important role in traditional Asian and West African medicine. In traditional Indian medicine various parts of the tree are used therapeutically, including for treatment of ascites, rheumatism, venomous bites, and as cardiac and circulatory stimulants. The roots, leaves and seeds are of particular importance in Ayurveda, and the uses of the roots, root bark, stem bark, stem exudates, leaves, flowers, and seeds treating a wide range of ailments

The flowers are also used as a tonic, diuretic and cholagogue. The leaves, rich in vitamin A and C, are considered useful in scurvy and respiratory ailments; they are also used as an emetic. The juice extracted from the leaves has strong antibacterial and antimalarial properties.

9. *Azadirachta indica*
Family: Meliaceae
Neem ingredients are applied in Ayurveda, Unani, Homeopathy, and modern medicine for the treatment of many infectious, metabolic, or cancer diseases. Different types of preparation based on plants or their constituents are very popular in many countries in disease management. In this vista, neem (*Azadirachta indica*), a member of the Meliaceae family, commonly found in India, Pakistan, Bangladesh, and Nepal, has therapeutics implication in diseases cure and formulation based on the fact that neem is also used to treat various diseases.

Azadirachta indica has complex of various constituents including nimbin, nimbidin, nimbolide, and limonoids and such types of ingredients play role in diseases management through modulation of various genetic pathways and other activities. Quercetin and ß-sitosterol were first polyphenolic flavonoids purified from fresh leaves and were known to have antifungal and antibacterial activity [36].

10. *Withania somnifera*
Family: Solanaceae
It **i**s an important medicinal plant of the Indian subcontinent. It is a woody shrub commonly known as "Indian ginseng" or "winter cherry." It is also known as ashwagandha in Sanskrit and asgandh in Urdu (Dhuley, 1998; Ziauddin et al., 1996). It was widely used alone or in combination with other herbs to treat numerous biological problems in humans. It possesses a wide spectrum of pharmacological properties, such as

antimicrobial, anti-inflammatory, antistress, antitumor, cardioprotective, and many more for use in the treatment of biological approaches. Thus, the plant and its steroidal components mitigate pathophysiological aspects of the disease; still, further studies are needed to prove the safety and efficacy of this compound in humans [37].

11. Cassia tora Linn.
Family: Leguminosae
It is a well-known plant widely distributed in India and other tropical countries. It is an annual shrub and grows in wild wasteland. Different parts of the plant (Leaves, seed, and root) are reputed for their medicinal value. It is well recognized in traditional medicine as laxative and is useful for treatment of leprosy, ringworm infection, ophthalmic, skin diseases and liver disorders. Several chemical compounds such as Anthraquinone glycosides, Naphthopyrone glycosides, Phenolic compounds, Flavonoids etc. have been isolated from this plant Properties are antibacterial, antifungal, and antioxidant, anti-inflammatory.
Antifungal Activity: The leaf extract has shown the significant antifungal activity to inhibit the growth of *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae* and *Trichophyton mentagrophyte*. It shows antifungal activity due to chrysophanol and chrysophanic acid- 9- anthrone and other anthraquinones such as emodine, physcion and rhein [38].

12. *Curcuma amada* (mango ginger)
Family: Zingiberaceae
It is a rhizomatous aromatic herb cultivated throughout India, Sri Lanka, Bangladesh and in many South-East Asian countries .The rhizomes are bitter, sweet, sour aromatic (a mixture of tastes, starting from bitter initially, turning to a sweet and then sour aromatic sensation), and cooling; used as an appetizer, carminative, digestive, stomachic, demulcent, febrifuge, alexeteric, aphrodisiac, laxative, diuretic, expectorant, anti-inflammatory and antipyretic and used in the treatment of anorexia, dyspepsia, flatulence, colic, bruises, wounds, chronic ulcers, skin diseases, pruritus, fever, constipation, hiccough, cough, bronchitis, sprains, gout, halitosis, otalgia and inflammations (Hussain *et al*., 1992; Warrier *et al*., 1994).
There is only very limited literature available on the pharmacological activity of the extract (Bhakuni *et al*., 1969; Rao *et al*., 1989). The rhizome extract of the plant exhibited a hyper-cholesteremic effect in rabbits (Pachuri and Mukherjee, 1970). The extract showed presence of an antibiotic principle with strong inhibitory activity on *Aspergillus niger* and *Trichophyton rubrum* (Gupta and Banerjee, 1972).

13. *Cassia angustifolia* (Senna)
Family Fabaceae
It is used as a laxative, and is a plant from the Fabaceae family. It includes hydroxyanthracene glycosides, also known as Senna Sennoside. In Brazilian traditional medicine, botanical species belonging to the genera *Senna* and *Cassia* are widely used as laxative, analgesic, and antifungal agents to treat ringworm and other fungal skin infections.

14. *Emblica officinalis or Phyllanthus emblica*
Family: Euphorbiaceae
Indian gooseberry or amla, is arguably the most important medicinal plant in the Indian traditional system of medicine, the Ayurveda and in various traditional folk systems of medicine in the Southeast Asia. The fruits are a rich source of vitamin C and are of dietary and culinary use in India.
In the traditional system of medicine, amla is used to treat a variety of ailments Scientific studies have shown that amla possesses antibacterial, antifungal, antiviral, antidiabetic, antiulcerogenic, antioxidant, antimutagenic, anti-inflammatory, immunomodulatory, antipyretic, analgesic, a antiatherogenic, gastroprotective, antianemia, anti hypercholesterolemia, wound healing, antidiarrheal, antiatherosclerotic, nephroprotective, neuroprotective and hepatoprotective properties [40].

15. *Citrus limon* (L.)
Family: Rutaceae
It is popularly known as the lemon tree is a species from the Rutaceae family, native to Asia. Lemon fruit is a rich source of nutrients, a key to a healthy diet, and provides health benefits. Lemons are an interesting source of flavonoids, vitamins, minerals, dietary fibers, essential oils, organic acids, and carotenoids. The nutritional composition, bioactive compounds, antioxidant properties, and other factors, such as antinutritional compounds and contaminants that may enter the fruit production chain, such as pesticides and heavy metals [41].
The main objective of our research work was to analyze the presence or absence of different phytochemicals in the selected fifteen medicinal plants used for antifungal activity.

MATERIALS AND METHODS

Collection of plant materials Fresh parts of fifteen medicinal plants were collected from different regions of Osmanabad and Beed districts of Maharashtra.

Srno.	Plant Name	Common Name	Parts used
1.	***Aloe vera***	Korphad	Leaves
2.	***Aegle marmelos***	Bael	Leaves
3.	***Psoralea coryfolia***	Bawarchi	Seed
4.	***Bauhinia variegata***	Kanchanar	Bark
5.	***Tinospora cordifolia***	Gulvel	Leaves
6.	***Eclipta alba***	Bhringraj	leaves
7.	***Withania somnifera***	Ashwagandha	Leaves
8.	***Azadirachta indica***	Kadulimb	Leaves
9.	***Moringa oleifera***	Shevga	Leaves
10.	***Cassia angustifolia***	Sonamukhi	Leaves
11.	***Curcuma amada***	Ambehalad	Rhizome
12.	***Cassia tora***	Tarvat	Leaves
13.	***Punica granatum***	Dalimb	Leaves
14.	***Phyllanthus emblica***	Amla	Leaves
15.	***Citrus limon***	Limbu	Leaves

Table. I Ethnobotanical information of selected medicinal plant species for phytochemical analysis.

The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. After drying, the plant materials were ground well using a mechanical blender into fine powder and transferred into airtight containers with proper labeling for future use.

Preparation of plant extracts

Solvent extraction Crude plant extract was prepared by the Maceration extraction method.
About 10gm of powdered plant material was placed in a stoppered container with 100ml Ethanol solvent and allowed to stand at room temperature. For a period of at least 3 days with frequent agitation until the soluble matter dissolved. The mixture was strained, the marc (the damp solid matter) was pressed, and the combined liquids were clarified by filtration. The filtrate was used for the phytochemical analysis.

Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods [12, [13],[14].

Test for proteins

Millon's test: Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test: Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates: Fehling's test Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Molisch's test: Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Iodine test: Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

Test for phenols and tannins: Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids

Shinoda test: Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after a few minutes which indicated the presence of flavonoids.

Alkaline reagent test: Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless in addition to a few drops of diluted acid which indicated the presence of flavonoids.

Test for saponins: Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for glycosides

Liebermann's test: Crude extract was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H_2SO_4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus. i.e., portion of glycoside.

Salkowski's test: Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani test: Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides (12 RNS Yadav and Munin).

Test for steroid: Crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

Test for terpenoids: Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A grayish colour indicated the presence of terpenoids

Test for alkaloids: Crude extract was mixed with 2ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Sr. No.	Plant Name	Proteins /a.a	carbohydrates	Phenols / Tannins	Flavonoids	Saponins	Glycosides	steroids	Alkaloids	Terpenoids
1	*Aloe vera*	+	+	+	+	+	+	+	+	+
2	*Aegle marmelos*	+	+	+	+	+	+	+	+	+
3	*Psoralea coryfolia*	+	-	+	+	-	-	+	+	+
4	*Bauhinia variegata*	-	-	+	+	+	+	+	+	-
5	*Tinospora cordifolia*	-	+	+	+	+	+	-	+	+
6	*Eclipta alba*	+	+	+	+	+	+	-	-	-
7	*Withania somnifera*	+	+	-	+	+	+	-	+	+
8	*Azadirachta indica*	+	+	+	-	+	+	+	+	+
9	*Moringa oleifera*	+	-	+	+	+	+	+	+	+
10	*Cassia angustifolia*	+	+	+	-	+	+	+	+	+
11	*Curcuma amada*	+	+	-	+	+	+	-	+	+
12	*Cassia tora*	+	+	+	+	-	+	+	+	+
13	*Punica granatum*	-	-	+	+	+	+	+	-	+
14	*Phyllanthus emblica*	-	+	+	+	-	-	-	+	-
15	*Citrus limon*	+	+	+	+	+	+	-	+	-

Results

+ = indicates presence of phytochemicals and - = indicates absence of phytochemicals.

Table. II Preliminary phytochemical analysis of medicinal plant species.

The phytochemical characteristics of fifteen medicinal plants tested were summarized in the table-1. The results revealed the presence of medically active compounds in the fifteen plants studied.

From the table, it could be seen that, proteins and amino acids, carbohydrates, phenols and tannins, flavonoids, saponins, glycosides, steroids, alkaloids, terpenoids were present in *Aloe vera* and *Aegle marmelos*. Carbohydrates, Phenols/tannins, flavonoids and alkaloids were present in *Phyllanthus emblica*. Carbohydrates, saponins, Glycosides were absent and Proteins, steroids, alkaloids, terpenoids were present in *Psoralea coryfolia* .Proteins, carbohydrates, terpenoids were absent in *Bauhinia variegata*. Proteins/amino acids, phenols/tannins, alkaloids, flavonoids, glycosides, saponins, steroids were present.

In *Tinospora cordifolia* proteins and amino acids, steroids were absent. Carbohydrate, Phenols/tannins, glycosides, saponins, alkaloids, flavonoids, terpenoids were present .Steroids, alkaloids and terpenoids were

absent and Carbohydrate, proteins, amino acids, phenols/tannins, saponins, flavonoids, glycosides were present in *Eclipta alba*.

Phenol/tannins and steroids were absent in *Withania somnifera* and *Curcuma amada*. Saponins were absent in *Cassia tora*. Flavonoids were absent in *Azadirachta indica* and *Cassia angustifolia*. In *Moringa oleifera* leaf extract Carbohydrates were absent. Proteins, carbohydrates and alkaloids were absent in *Punica granatum*. In *Citrus limon* steroids and alkaloids were absent.

Discussion:

Phytochemical analysis concluded on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [12]. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [16]. Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds [18], [19].

Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. [20]. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against a wide array of microorganisms in vitro. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [21]. They also are effective antioxidants and show strong anticancer activities [22], [23], [24]. The plant extracts were revealed to contain saponins which are known to produce inhibitory effects on inflammation [25]. Steroids have been reported to have antibacterial properties [27]. Alkaloids have been associated with medicinal uses for centuries and one of their cytotoxicity [29]. Several workers have reported the antispasmodic and antibacterial [31], [32] properties of alkaloids. The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit.

Conclusion:

The selected fifteen medicinal plants are the source of the secondary metabolites i.e., alkaloids, flavonoids, terpenoids, reducing sugars. Medicinal plants play a vital role in preventing various diseases. The anti-inflammatory, anticancer, antiviral, antibacterial and antifungal activities of the medicinal plants due to presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for manufacturing of new drugs. The phytochemical analysis of medicinal plants are also important and have commercial interest in both research institutes and pharmaceutical companies for the manufacturing of new drugs and biofertilizers for the treatment of various plant diseases. Thus we hope that the important phytochemical properties identified by our study in the local plant of Osmanabad and Beed district will be helpful in copping different diseases of this particular region.

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