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SCREENING OF PHYTOCHEMICALS & DETERMINATION OF ANTIOXIDANTS CONTENT AND ANTIMICROBIAL ACTIVITY IN MEDICINAL PLANTS OF KALYAN

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ABSTRACT

Medicinal plants are composed of phytochemicals that improve the physiological balance of human beings and also used for treatment of various health ailments and chronic diseases. The phytoconstituents in different locally available plant parts in Kalyan viz. Tulsi leaves and stem, Ajwain & Cloves drawn out as distilled water extracts were subjected to phytochemical screening which affirmed the presence of large array of phytochemicals. The important bioactive constituents (flavonoids ,ascorbic acid, phenolic compounds) of these plants were determined. The aluminum chloride colorimetric method was used for determination of Total Flavonoid Content (TFC). Tulsi leaves and stem, Ajwain and cloves contained flavonoids as Quercetin equivalents amounting to 37.966±0.42, 77.525 ±0.727 and 27.525±0.370 & 39.987±0.463 mg/g of QE respectively. The 2, 4 Dinitrophenyl hydrazine method was used for determination of total ascorbic acid content. Ascorbic acid content in Tulsi leaves and stem, Ajwain and cloves was found to be 34.909±0.72, 44.49±0.89, 49.94±0.77, 43.032±0.45 mg/g dried plant sample respectively. Total phenol was estimated as tannic acid equivalent using the Folin-Ciocalteu phenol reagent method and was found to be 243.45±1.045, 235.694±1.056, 327.611±0.690, 317.59±0.917 mg/g respectively of TA equivalents in the extracts. Antimicrobial activity of the aqueous and ethanol extracts was investigated against selected microorganisms. Aqueous extracts did not show any antimicrobial activity against the test cultures used. However, ethanol extract showed antibacterial activity against four test cultures used with Clove extract showing maximum activity. The extracts also exhibited antifungal activity against *Candida albicans.*

KEYWORDS: Medicinal plants, Phytochemicals, Flavonoids, Ascorbic acid, Antimicrobial activity.

INTRODUCTION

Medicinal plants were recognized to contain a few of natural compounds having definite physiological action on the human body.[1] The availability of plant secondary metabolites phytochemicals present in leaves, stems, bark or roots of vegetables and other plants is related to their antioxidant potentials and medicinal properties of the plants and their extracts.[2] For decades, serious attention has been paid to the use of traditional medicines and plant drugs against numerous diseases,[3],[4] as they are safer with little or no side effects.[5] Identification of phytoconstituents in the plant material helps to predict the potential pharmacological activity of that plant.[6]

Phytochemicals are bioactives and function to protect plants against invasion, disease, and infection. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, tannins etc. In India, phytochemicals, as well as medicinal plants, have remained the most abundant source of healthcare and life improvement since very long.[7] Ayurvedic, Unani and Siddha medico-therapeutics have played a very important role in Indian society since ancient times. Now in the modern era, about 24%–27% drugs are derived from plant sources. India has one of the richest plant biodiversity in the world, especially with Western Ghats and Himalayas being regions rich in plant biodiversity within the country. About 7500 plant species out of 43,000 are recorded in various medicines, and ~1700 species are acknowledged in Ayurvedic literature.[8] In India, phytochemicals are not limited to medicinal use only, but they have been used in cosmetics, health and hygiene, fragrance, and food supplements.

In view of this background the present study aimed to investigate preliminary phytochemical analysis of the water and alcoholic extract of Tulsi (Leaves & Stem), Ajwain and cloves and quantification of Flavonoids, Ascorbic acid and tannins in the extracts.[9]

Flavonoids are widespread natural antioxidants They can be found in many plants,[9][11] tea, beer, juices, and wines.[12],[13] Flavonoids have multiple effects on humans. They have anti-inflammatory, antihistaminic, antioxidant, antiedema, and anticancer effect; they stabilize cell membranes, inhibit aging processes and have a positive effect on the function of the cardiovascular system.[12][14] Ascorbic acid C is the most abundant water-soluble compound working in one-electron reactions, and it is an essential micronutrient and a key element for the metabolism of almost all living organisms. In humans, it has numerous functions, mainly acting as an antioxidant and cofactor for mono-oxygenases and dioxygenases.[15] Ascorbic acid is an essential element of plant and animal antioxidant systems, which can be defined as complex redox networks, including metabolites and enzymes, with mutual interactions and synergistic effects.[16] Ascorbic acid deficiency leads to scurvy. Characteristic features of scurvy includes, spongy swollen bleeding gums, dry skin, open sores on the skin, fatigue, impaired wound healing and depression.[17]

Phenolic compounds are the most abundant secondary metabolites in plants, playing a key role in pigmentation, growth and reproduction of the plant, together with resistance to pathogens and predators. This is largely due to their phytoalexin properties and potent astringency which have been shown to provide anti-allergic, anti-inflammatory, antioxidant, hepatoprotective, antiviral, and anticarcinogenic activities too.[18] Ajwain (*Trachyspermum ammi*) is an ancient spice with a lot of medicinal properties and the major phytochemical reported is a phenolic compound. These compounds can act as antibacterial and antifungal agents. The results indicate that ajwain extract can be used in further medicinal applications and also as a preservative action.[19] Clove is a recognized herbal medicine in Ayurveda and is primarily used as a home remedy for toothache. It also provides relief from cold and sore throat due to its antibacterial properties.[20] Studies have shown that eugenol and carvacrol are the phenolic compounds that are the major components of essential oil present in clove buds.[21] The most studied plant with medicinal properties is Tulsi (Holy basil)-(*Ocimum tenuiflorum)*. Traditionally tulsi has been used in the treatment of a number of ailments and diseases.[22] Studies have indicated that tulsi contains a large number of bioactive compounds like eugenol, rosmarinic acid, luteolin to enlist a few. These are responsible for the antimicrobial and other activities reported by the plant.[23]

MATERIALS AND METHODS

Samples

Tulsi leaves, Tulsi stem, Ajwain, Cloves were purchased from a nursery at Kalyan.

Apparatus

All glassware used for the experimental purpose was made up of Pyrex or Borosil glass. The burette, pipette and standard flasks were calibrated by the method described by Vogel.[24]

Instrument

The absorption measurements were carried out on a visible spectrophotometer, model LMSP-V320, LABMAN, using 1-cm matched glass cells. The spectrophotometer was calibrated by measuring the absorption spectra of potassium chromate in potassium hydroxide solution and that of potassium permanganate in sulphuric acid solution.[25]

Chemicals

10% AlCl3, Quercetin standard, Ascorbic acid, Tannic acid, Folin-Ciocalteu reagent, 2,4 dinitrophenyl hydrazine.All the chemicals used were of A.R. grade.

For Microbiological analysis the media was procured from Himedia labs. The cultures were laboratory strains *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Corynebacterium diphtheria, Candida albicans.*[19] The zone was inhibition was reported in terms of determination of standard deviation between the mean values of the zone of inhibition which was used to measure the antimicrobial activity. All the tests were done in triplicates.[26]

METHODS

1. Screening of Phytochemicals for Tulsi (Stem & Leaves), Ajwain & Cloves

Preparation of plant extracts: Tulsi leaves, Tulsi stem, Ajwain & Cloves were dried in a microwave oven at 900 watts at 525 degrees for 3 min .Tests were carried out using a distillation method for all samples. 1 gm of each sample in 100 ml of distilled water was further refluxed for 2.5 hours in distilled water to obtain aqueous extract. For antimicrobial activity, ethanolic extract was prepared by boiling the samples (0.5g) in ethanol (50 ml) for 20 minutes. Qualitative phytochemical examinations for all the samples were carried out as per the standard methods.[27][31]

2. Quantitative determination of antioxidants

a) Determination of Flavonoid content

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content (TFC) of the sample. Calibration curve was plotted in the range of 10-100 ug/ml of quercetin and absorbance of the complexes measured at 415 nm. Quercetin was used as the standard to plot the calibration curve for the methods.[32]

Standard solution of quercetin was prepared by dissolving 10mg of Quercetin in 10 ml of methanol .Different concentrations were prepared after appropriate dilutions to get 20,40 ,60,80,100 ug/ml of solution. Absorbance was taken at 415 nm against the suitable blank. Flavonoids were determined in different plant samples obtained after extraction in methanol by using reference method. The assay was determined using 1 ml of each extract stock solution and diluted appropriately. All the prepared solutions were filtered through Whatmann filter paper before measuring their absorbance.

b) Quantitative estimation of Ascorbic acid

Standard solution of ascorbic acid was prepared by dissolving 10 mg of ascorbic acid in 10 ml of distilled water and different concentrations were prepared after appropriate dilutions to obtain 20 to 100 ug/ml of solution. The 2,4-dinitrophenyl hydrazine method was used for the determination of the total ascorbic acid content of the sample. Calibration curve was plotted in the range of 10-100 ug/ml of ascorbic acid. The absorbance of the complexes was measured at 521 nm.[33]

c) Quantitative estimation of total phenolic content

Standard solution of tannic acid was prepared by dissolving 10 mg of tannic acid in 10 ml of distilled water and different concentrations were prepared after appropriate dilutions to obtain 20 to 400 ug/ml of solution. The Folin-Ciocalteu method was used for the determination of total phenolics in the samples and the amount of total phenols were calculated from the calibration curve as tannic acid(TA) equivalents, as given in the literature.[34]

d) Statistical Analysis

Data were analyzed using Microsoft Excel and reported as mean±standard deviation of triplicate determination.

2. Microbiological analysis

Antimicrobial activities of the prepared plant extracts were tested against representative gram positive, gram negative and yeast using agar cup diffusion method. To check antibacterial and antifungal activity, Mueller Hinton agar and Sabouraud dextrose agar medium were used. *Escherichia coli*, *Pseudomonas aeruginosa* , *Staphylococcus aureus* and *Corynebacterium diphtheriae* and yeast *Candida albicans* were selected as test cultures for the present study.[22] Suspensions of pure cultures were prepared in sterile saline having absorbance of 0.1 at 530 nm. 0.5 ml of each suspension was seeded separately into a 25 ml sterile medium at around 40°C and poured into the sterile Petri plates aseptically. After solidification, six wells were made aseptically in each plate using an 8 mm sterile cork borer. 50µl of each alcoholic test extract was introduced into the wells. The plates were incubated at 37ºC for 24 to 48 hours. The test compound under investigation diffuses radially from the well into the surrounding medium containing the test culture. As it diffuses far from the well, the concentration of the test compound decreases, thus a gradient of the test compound is established, with the highest concentration of compound at the well. The sensitive test culture will start growing in the medium from the point where the concentration of the test compound is non-inhibitory. Thus, inhibitory effects of the test compound can be seen as a 'zone of inhibition' of the test organism surrounding the well. The extent of the zone of inhibition is an indication of the effectiveness of compounds' inhibitory properties. The larger the zone of inhibition, the better is the antimicrobial property of that compound. The zone of inhibition was measured using the Hi-media zone scale. Standard antibiotic streptomycin (S) at a concentration of 100 mcg/ml was prepared in sterile distilled water and 50µl was added to one of the agar cups as a positive control. Similarly, 50µl ethanol (E) was added in the other cup as a negative control, since the plant extracts were prepared in ethanol, it was necessary to check that ethanol is not inhibitory to the test cultures. All the assays were performed in triplicates.[19]

RESULTS AND DISCUSSION

Results

1. Screening for presence of Phytochemicals in Tulsi (Stem & Leaves), Ajwain & Cloves

The phytochemical quantitative composition of Tulsi Leaves & stem, Ajwain and Clove are shown (Table1).

Table 1: Screening of Phytochemicals for Tulsi (Stem & Leaves), Ajwain & Cloves.

Phytochemical	Tulsi Leaves	Tulsi Stem	Ajwain	Clove
Alkaloids	+	-	-	+
Carbohydrates	-	-	-	-
Glycosides	+	+	-	-
Flavonoids	+	+	+	+
Resins	-	-	-	-
Saponins	+	+	-	-
Steroids	-	-	-	-
Terpenoids	+	-	+	-
Tannin	+	+	+	+
Inorganic acid	-	-	+	-
Organic acid	+	+	+	-
Phenolic	+	+	+	+
Amino acid	-	-	-	-
Proteins	-	-	-	-
Oils & Fats	-	-	+	-
Coumarins	-	-	+	-
Phlobatannins	-	-	-	-
thraquinone	-	-	-	-

+ Indicates presence of phytochemicals, - Indicates absence of phytochemicals

Flavonoids, Tannins phenolic contents, organic acids were detected in all the extracts, whereas carbohydrates resins, steroids amino acids, proteins, phlobatannins, anthraquinones were not detected as shown.

2. Quantitative analysis of bioactive compounds in Plant extracts

a) Quantification of flavonoids

Flavonoids were quantitatively estimated in the plant extracts by method cited in the reference. The total flavonoid content (TFC) was determined as Quercetin equivalent (QE mg/g of extracted compound). All the readings were recorded in Triplicate. (Fig. 1; Table 2). For quantification of flavonoids, complexation with $AlCl_3$ shows satisfactory performance according to several studies described in the literature. The results showed that all plant extracts tested found to contain comparable high total flavonoids as rich sources of bioactive compounds and thus justifies the use for human health benefits. Maximum flavonoids content was in tulsi stem as 77.746 mg/g.

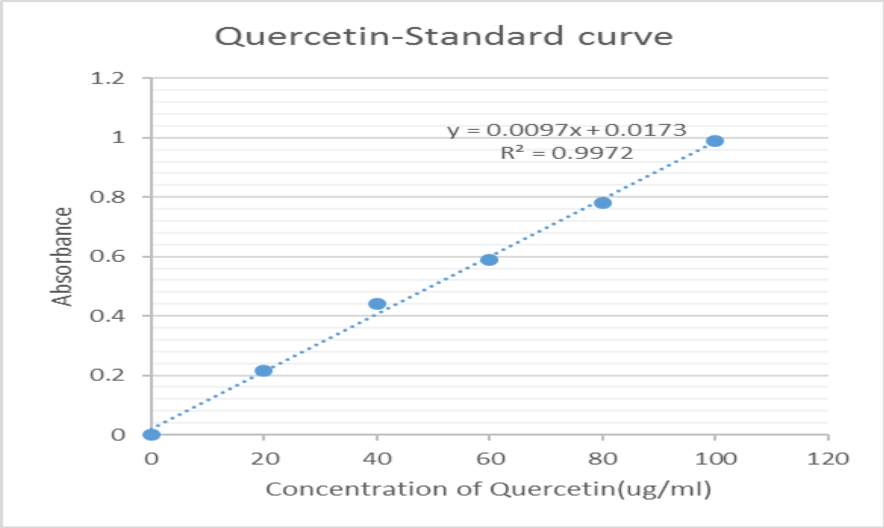

Fig .1: Calibration curve of Quercetin (ug/ml).

b) Quantification of Ascorbic acid

The quantification of ascorbic acid after complexation with 2,4 DNP reagent shows satisfactory performance according to several studies described in the literature. Maximum ascorbic acid was found in Ajwain as 49.94 mg/g .The results showed that all plant extracts tested found to contain total ascorbic acid as rich sources of bioactive compounds .(Fig-2,Table2).

c) Quantification of total phenolic content (PC)

These plants show high constitutions of phenols and phenolic compounds which imply that they may be used as antimicrobial agents. Table 2 shows that Ajwain has high amounts of phenol 327.611mg/g extract. Phenols and phenolic compounds are greatly used in skin infections and other wounds treatment and also for healing, when compared to other bactericides. (Fig-3, Table2)

Table 2: Flavonoid contents, Ascorbic acid & total phenolic content in plant extracts.

PLANT EXTRACTS	TFC as QE (mg/g)±SD	Ascorbic acid (mg/g)±SD	PC as TA (mg/g)±SD
Tulsi Leaves	37.966±0.427*	34.909 ±0.72*	243.45 ±1.045*
Tulsi stem	77.746±0.272*	44.49 ±0.89*	235.694±1.056*
Ajwain	27.525±0.370*	49.94 ±0.768*	327.611±0.690*
Cloves	39.987±0.463*	43.032 ±0.454*	317.59±0.917*

*=SD for triplicate analysis

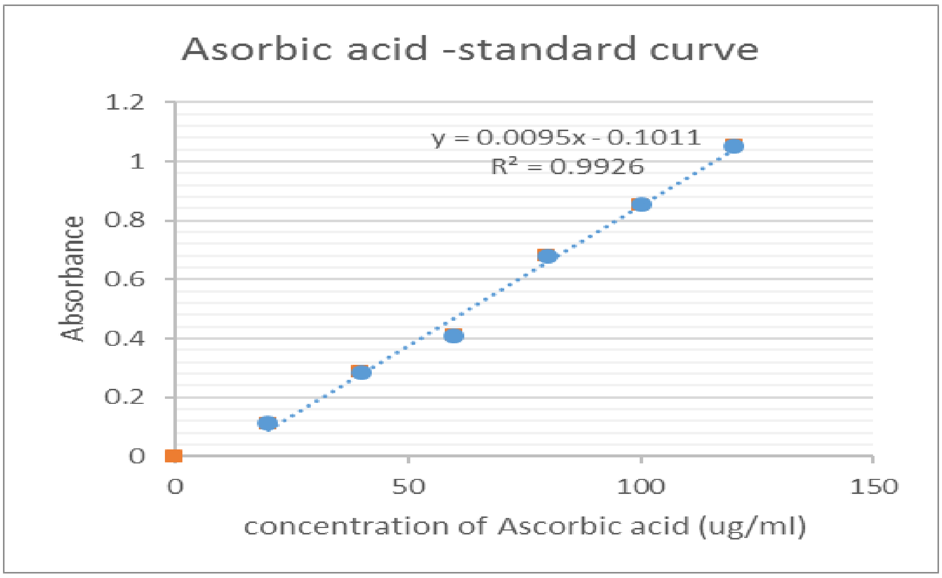

Fig: 2: Calibration curve of Ascorbic acid.

d) Quantification of total phenolic content

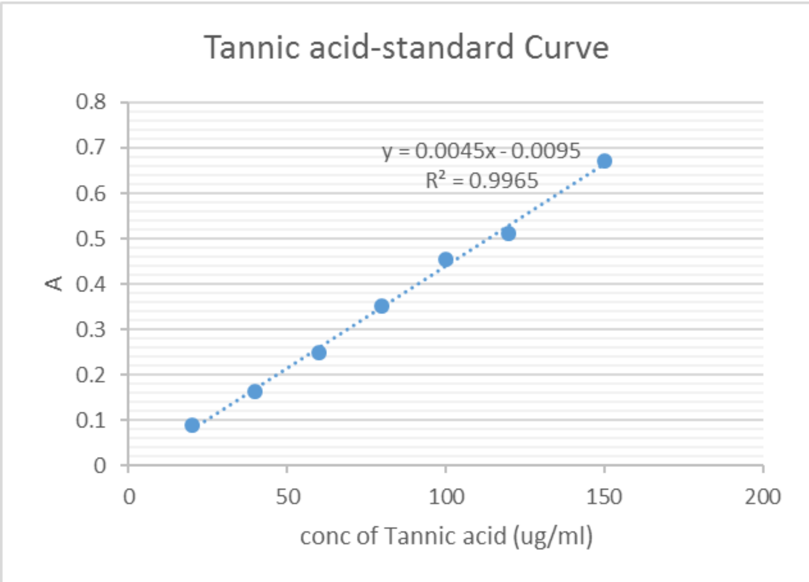

Fig 3: Calibration curve of tannic acid.

3. Microbiological analysis

Antimicrobial analysis of the phytochemical extracts by agar cup method revealed antimicrobial activity against all the test organisms selected. The maximum zone of inhibition was given by Clove extract against *Staphylococcus aureus*. (Figure 4)

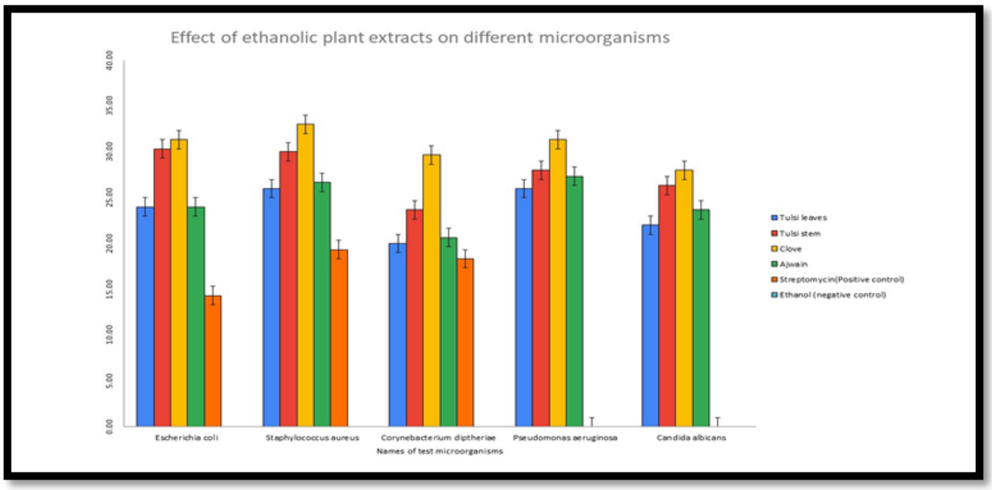

Fig 4: Effect of ethanolic plant extracts on different microorganisms.

Ajwain extract inhibited *Pseudomonas aeruginosa*. Tulsi stem extract gave maximum antifungal activity against *Candida albicans*. All the results were compared with standard antibiotic streptomycin (100 mcg/ml) and results indicated that the alcoholic extracts were showing more inhibition than the antibiotic used against the selected test cultures. (Figure 5)

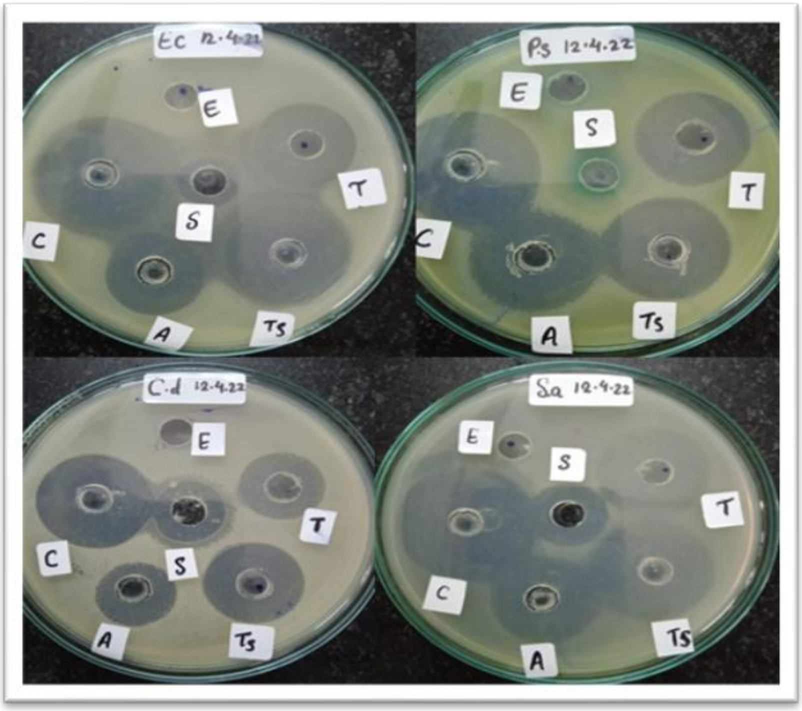

Fig 5: Antimicrobial effect of ethanolic plant extracts on different microorganisms.

DISCUSSION

The medicinal plants produce a large number of secondary metabolites which since ancient years have proven to have diverse functions. They are used in therapy for both human and veterinary, and are active metabolites in many formulations.[35] Different phytochemicals have an extensive range of activities, which helps to enhance the immune system and give resistance against long term disease to protect the body from harmful pathogens. The main purpose of this study was to examine and investigate the phytochemicals present in the selected medicinal plants commonly used in Kalyan, India. Flavonoids, ascorbic acid and phenolic compounds are the most important bioactive components of plants, which were studied quantitatively. The results showed that all plant extracts tested contained total flavonoid content, ascorbic acid and total phenolic content.

The recent rise of microbial resistance to antibiotics has raised serious concerns for treating infections. Scientists are exploring the potential of phytochemicals to treat infection caused by drug resistant pathogens. Phytochemicals are plant-derived chemicals that are chemically diverse. These compounds have demonstrated beneficial advantages in terms of antioxidant, antibacterial, and antifungal activities.[36]

The extract of tulsi showed the presence of alkaloids, Flavonoids, Glycosides, organic acids. In a similar study by Bohra et al., similar phytochemicals have been reported. In the present study, the ethanolic extracts of Tulsi leaf, Tulsi stem, Clove bud and Ajwain showed good antibacterial activity against five selected test cultures. The five test cultures, were selected on the basis of their well-known pathogenesis. *P. aeruginosa*, gram-negative bacteria can cause multiple infections in man that vary from local to systemic and from benign to life threatening.[37] This organism is known for both intrinsic and acquired resistance to many classes of antimicrobial agents.[38]*E. coli*, a gram-negative bacterium that normally lives in the intestines of both healthy people and animals is normally harmless. However, under certain condition, these can turn into opportunistic pathogens. Certain strains of *E. coli* are also reported to cause diarrhea. *S. aureus* are Gram-positive bacteria and are responsible for the most common bacterial infections in humans viz., bacteremia, infective endocarditis, skin and soft tissue infections, osteomyelitis, septic arthritis, prosthetic device infections, pulmonary infections, gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections.[39] *C. diphtheriae* are gram-positive rod-shaped bacteria and is the causative agent of diphtheria which is most commonly a pediatric infection of the upper respiratory tract and causes fever, sore throat, and malaise.[40] *Candida albicans* is an opportunistic pathogenic yeast. They are the causative agents of oral, gastrointestinal, and vaginal candidiasis.[41]

Ethanolic extract of tulsi stem demonstrated a better antimicrobial activity as compared to tulsi leaf extract. Furthermore, the antibacterial activity was similar for both gram positive and gram negative isolates. The antifungal activity of both extracts was slightly less than antibacterial activity. The essential oil extracted from tulsi is well known for its antibacterial properties.[42] The results of our study are in agreement with those reported by Mishra and Mishra.[43] who reported antibacterial activity of Tulsi Extract against both gram positive and gram begative bacteria. In a similar study carried out by Mallikarjun S et al, the results have indicated antimicrobial activity of tulsi leaves and inflorescence against periodontal pathogens. We have also reported similar results from tulsi stem extract. The stems of tulsi are a part of the samples i.e. the leaves and the inflorescence.[44]

Clove extract tested showed the presence of alkaloids, phenolics, tannins and flavonoids. It also has the presence of ascorbic acid which helps in the antioxidant property.[45] The ethanolic extract of clove showed a significant antibacterial and anti fungal activity in comparison to the selected antibiotic (100 mcg/ml streptomycin). As reported by Sewani & Quereshi and Abdullah *et al*., antibacterial activity was not seen in the aqueous extract indicating that the antimicrobial constituents of clove are more soluble in ethanol than water.[46], [47] The clove essential oil, especially eugenol is thought to destroy the cell wall and cell membrane, which cause leakage of intracellular constituents. Further, it is also hypothesized that the constituents of essential oils enter inside the cells and inhibit DNA and protein synthesis. Thus, antimicrobial activities of essential oils of clove are primarily due to molecular mechanism than just physical damage.[48]

Ajwain extract was found to be rich in oils and fats, flavonoids, phenolics, organic and inorganic acids, terpenoids and coumarin. Ajwain ethanolic extract gave promising results for both antibacterial and antifungal studies. Sharma has reported the validation of different solvents like hexane and methanol and its effect on antimicrobial activity.[49] The GC-MS analysis of the extracted ajwain oil showed presence of thymol, as its major component (71.06%) along with o-Cymene (3.37%), γ-Terpinene (3.83%), 2-methyl-5-(1-methylethyl)-phenol (0.51%). It is these constituents of Ajwain that are likely to be responsible for antimicrobial activities.[19]

Thus, studies using other essential oils also reported variable results, depending on the oil and analytical method selected. Thielmann et al. (2019), have demonstrated variable antibacterial activity by screening essential oils against a range of food borne gram-positive and negative bacteria.[50] Several investigators reported that Gram-positive bacteria are more sensitive to essential oils in general than Gram-negative bacteria. [51] The results are variable which could be due to the seasonal variation, the variety of the plant material and the extraction methods used.

CONCLUSION

The analysis of the content of main active components in raw materials and phytomedicines is an essential step to evaluate the quality of products and validate the efficacy and safety of their therapeutic use.

The present study of qualitative study of Tulsi, Ajwain, clove showed that it contains phytochemicals mainly saponins, reducing sugars, flavonoids, phenols, tannins & terpenoids. These phytochemicals act as a source of useful drugs and also to improve human health as a result of the presence of these various phytochemicals as they have the potential to be powerful antioxidant/antimicrobial agents. Ayurveda, is a traditional herbal medical system which has originated in India. The ancient wisdom in this traditional system of medicine is still not exhaustively explored. Thus, the rich knowledge of traditional systems of medicine can be a potential source for the development of new herbal drugs.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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