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PHYTOCHEMICAL INVESTIGATION AND ANTIMICROBIAL SCREENING OF MEDICINAL PLANTS OF TUMAKURU DISTRICT, KARNATAKA, INDIA

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ABSTRACT: The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not endorsed to a single compound but a combination of the metabolites. The medicinal actions of plants are exclusive to a particular plant species or group, unswerving with the perception that the combination of secondary products in a particular plant is taxonomically distinct. Preliminary phytochemical screening, antibacterial and antifungal potential of four medicinal plants was the aim of our study. Qualitative phytochemical screening of these plants confirmed the presence of various phytochemicals like flavonoids, steroids, terpenoids, saponins, phenols, tannins and anthroquinones gave the plant extracts its potential against various disease. Among the phytochemicals screened steroids were found abundant followed by flavonoids. Significant zone of inhibition was observed in methanol extract of C. *asiaticum* against *S. flexneri* (16± 0.91mm) and (12± 0.54mm) inhibition of *Proteus* sp. in ethanolic extracts. *C. martinii* showed 16± 0.78 mm of inhibition against *Proteus* sp. in methanol extract and ethanol extract showed (12± 0.49 mm) against *S. flexneri.* Further ethanol extract of *C. asiaticum* proved efficient against *T.viride* showing (12±0.87 mm) and (9±0.63mm) of inhibition in methanol extracts against *Cladosporium* sp. Methanol extracts *C. martini* showed similar inhibition zone of (8± 0.27 mm) against *T.viride* and (8±0.21mm) against *Cladosporium* sp. Also methanolic and ethanoic extracts of *C. tuberosa and T. indica* showed partial inhibitory effect with test microorganisms. The results lend scientific credence to justify the use of these plants against microbial infections.

KEYWORDS: Phytochemical Screening, Antibacterial Activity, Medicinal Plants, Methanol extract, C. *asiaticum, C. martini, S. flexneri., T. viride.*

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1. INTRODUCTION

Plants role is twofold in the development of medicines and served as a natural blue print for the development of new drugs. Medicinal plants represent a rich source of antimicrobial agents [1]. Their resources serve as a potential source for wet finish application on the textiles, for treatment of skin wounds, antifungal, anti-microbial finish [2]. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs and 80% of world population is reliant on traditional medicine and a major part of traditional therapies involves the use of plant extracts or their active constituents. Yet a scientific study to determine their antimicrobial active compounds is a comparatively new field [3-4]. The secondary metabolite of the plants is very essential commercially and has more concentration in pharmaceutical companies for the manufacture of the new drugs for curing of several diseases [5]. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmaceutical. Plant parts such as leaves, roots and bark are used for the therapeutic purposes and as well serve as precursors for the synthesis of useful drugs due to their ethno-medical importance in nature. The medicinal potentials of these plants could be traceable to the bioactive phytochemical ingredients that are pertinent for the physiological action on the human body [6]. General screening of plants such as antimicrobial assay was chosen as the simple, cheap and fast *in vitro* bioassay [7]. The capability of higher plants as hotspot for new medications is still to a great extent unexplored. Among the assessed 250,000-500,000 plant species, just a diminutive rate has been researched. Phytochemicals and the division submitted to organic or pharmacological screening is considerably little. In this way, any phytochemical examination of a given plant will uncover just an extremely contract range of its constituents. Verifiably pharmacological screening of mixes of normal or engineered starting point has been the wellspring of incalculable restorative operator. Nature is a peerless source of structures of high phytochemical diversity and many of them possessing interesting biological activities and medicinal properties. In the context of the worldwide spread different diseases such as AIDS, chronic diseases and a variety of cancers, an intensive search for new lead compounds for the development of novel pharmacological therapeutics is extremely important. With the present information it is difficult to establish clear functionality and structural activity relationships regarding the effects of phytochemicals in biological systems activity. This is largely due to the occurrence of a vast number of phytochemicals with similar chemical structures, and to the complexity of physiological reactions. Moreover, the given number of phytochemicals isolated so far, nature must still have many more in store. With the advances in synthetic methodology and the development of more sophisticated isolation and analytical techniques, many more of these phytochemicals should be identified [8]. Substances derived from plants have recently being of great interest due to their versatility. These substances in the plant which enhance their usefulness

globally are classified as phytochemicals [9]. Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols flavonoids etc, which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc. The beneficial medicinal effects of plant materials typically result from the combination of these secondary products. Phytochemicals are known to possess antioxidant, antibacterial, antifungal, antidiabetic and anti-inflammatory effects [10].

2. MATERIALS AND METHODS

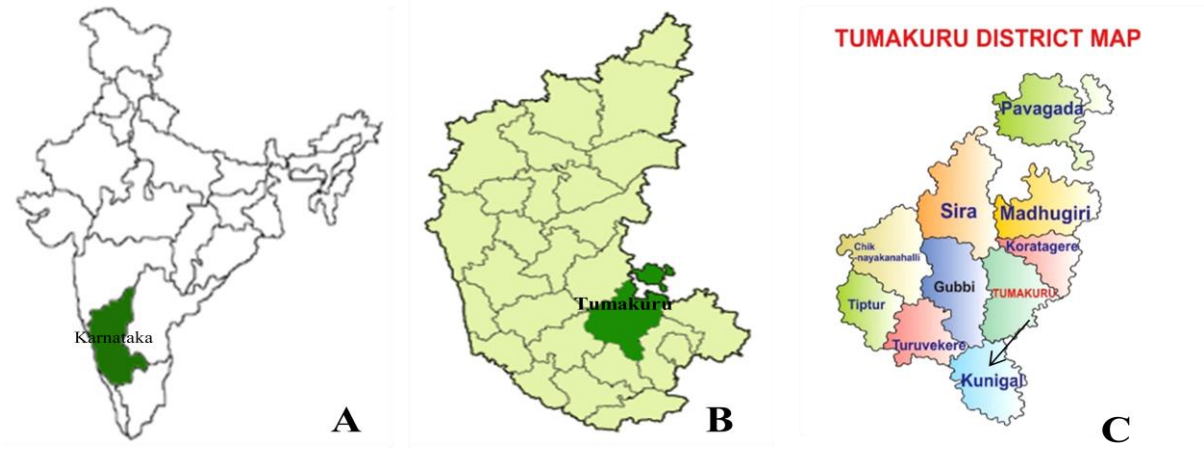

Fig 1: Map showing geographical location of

A) India - Karnataka; B) Karnataka - Tumkuru; C) Tumkuru District-Kunigal Tq.

Sl. No.	Common name	Botanical name	Family	Parts used	Traditional uses
1	Adumuttada gida	*Tylophora indica*	Apocyanaceae	leaves	Bronchial asthma, Jaundice, inflammation, muscle relaxant, dermatitis & rheumatism, anticancerous & antiamoebic
2	Palmorosa	*Cymbopogan martinii*	Poaceae	grass	Insecticide, flu, anti-inflammatory, analgesic, malaria, cosmetics.
3	Sahyadri - dew grass	*Cyanotis tuberosa*	Commelinaceae	roots	Rheumatism, stimulating blood circulation & arthritis.
4	Vishamoongali	*Crinum asiaticum*	Amaryllidaceae	bulbs	Tonics, laxatives, expectorants, urinary infection, skin disorders, nausea & vomiting

Fig 2: A- *Tylophora indica*; B- *Cymbopogan martinii*; C- *Cyanotis tuberosa*; D- *Crinum asiaticum*

2.1 Collection of plant material: The fresh disease free leaves of *Tylophora indica*, grass of *Cymbopogan martinii*, fresh roots of *Cyanotis tuberosa* and bulbs of *Crinum asiaticum* were collected from Sigepalya village of Huliyur Durga Hobli, Kunigal taluk of Tumkuru district (Fig.1). The plants were selected from the local area based on their basic information available. Leaves, grass, roots and bulbs were washed in tap water thoroughly, shade dried and powdered (Fig.2; Table.1). The plants were identified and authenticated from P. G. Department of Botany, Maharani's Science College for Women, Mysuru. The voucher specimen of the plants numbers *viz. T. indica* (MSCWM/ PG/ 2018-128), *C. martinii* (MSCWM/ PG/ 2018-225), *C. tuberosa* (MSCWM/ PG/ 2018-157) and *C. asiaticum* (MSCWM/ PG/ 2018-134) have been kept in the department for further studies.

2.2 Preparation of plant extracts

Aqueous Extract: 10 g of shade dried, powder of leaf material of all selected plant species were macerated with 100 ml of sterile distilled water in a blender for 15 min. The macerate was first filtered through double layered muslin cloth and then filtrate was centrifuged at 4000 rpm for 30 minutes at room temperature. Supernatant was filtered through Whatman No.1 filter paper and the supernatant was made up to make the final volume one-fourth of original volume which was heat sterilized at 121ºC for 20 minutes. The extract was preserved aseptically in brown airtight bottles and stored at 4 ºC for further use.

Methanol Extract: 10 g of shade dried, powder of leaf material of all selected plant species were macerated with 100 ml of methanol kept on a rotary shaker at 190 - 220 rpm for 24 hrs. The filtrate was first filtered through double layered muslin cloth and then filtrated through Whatman No. 1 filter paper and the supernatant was made up to make the final volume one-fourth of original volume which was heat sterilized at 121ºC for 20 minutes. The extract was preserved aseptically in brown airtight bottles and stored at 4 ºC for further use.

Ethanol Extract: 10 g of shade dried, powder of leaf material of all selected plant species were macerated with 100 ml of ethanol kept on a rotary shaker at 190 - 220 rpm for 24 hrs. The filtrate was first filtered through double layered muslin cloth and then filtrated through Whatman No. 1 filter paper and the supernatant was made up to make the final volume one-fourth of original volume which was heat sterilized at 121 ºC for 20 minutes. The extract was preserved aseptically in brown airtight bottles and stored at 4 ºC for further use. All the extractions were carried out following the procedures [11].

2.3. Phytochemical Analysis

The selected plants were subjected to preliminary phytochemical analysis to trace the presence of phytochemicals like flavonoids, steroids, terpenoids, saponins, phenols, tannins and anthroquinones etc. Preliminary phytochemicals analysis was carried out for all the extracts as per standard methods [12 -13].

Detection of Flavonoids

a) Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicated the presence of flavonoids.

b) H_2SO_4 test: Extracts were treated with few drops of H_2SO_4. Formation of orange color indicated the presence of flavonoids.

Detection of Steroids

Salkowski's test: The formation of red color in the lower chloroform layer when 0.2 g of organic extracts (Petroleum ether, $CHCl_3$/MeOH (1:1) and MeOH crude extracts) dissolved in 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added to it which indicated the presence of steroids.

Detection of Terpenoids: 0.2 g of the organic extract was dissolved in 2 ml of $CHCl_3$ and evaporated to dryness. 2 ml of conc. H_2SO_4 was then added and heated for about 2 min. Development of a grayish color was indicated the presence of terpenoids.

Detection of Anthraquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHC_{l3} was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicated the presence anthroquinones.

Detection of Phenols

a) Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicated the presence of phenol.

b) Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Detection of Saponins

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

Detection of Tannins

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. Formation of dark green color indicated the presence of tannins. All the phytochemical analysis was carried out following the procedures [14].

2.4 Growth and maintenance of test microorganisms for Antimicrobial activity

The identified pathogenic bacteria *Escherichia coli, Streptococcus pneumoniae, Shigella flexneri, Proteus* sp. and fungi *Cladosporium* sp. and *Trichoderma viride* were obtained from PG Department of Microbiology, Maharani's Science College for Women, Mysuru, Karnataka, India. The bacterial cultures were grown and maintained on Nutrient Broth (NB) at 37 ºC, while the fungal cultures were maintained on Potato Dextrose Agar (PDA) slants and incubated at 27 ºC for further studies.

Preparation of microbial inoculum: *E. coli, S. pneumoniae, S. flexeri, Proteus* sp. were pre-cultured in nutrient broth over night in a rotary shaker at 37^0 C and centrifuged at 10000 rpm for 5 min and the pellets obtained was suspended in double distilled water and the cell density was standardized Spectrophotometrically (A_{610} nm). The fungal inoculum was prepared from 7-10 day old cultures grown on potato dextrose agar medium. The petridishes were flooded with 8-10 ml of distilled water and the culture was scraped aseptically using sterile spatula. Spore density of each fungus was adjusted with spectrophotometer (A_{595}nm) to obtain a final concentration of approximately 10^5 spores/ ml [15].

2.5 Agar-Well Difussion Method

Antibacterial Activity: Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. Nutrient agar plates were swabbed with 24 hr old culture of selected bacteria with sterile cotton swabs. 10 mm wells were made in each NA plates using sterile cork borer. 100 µl of each extract were added to the wells by using micropipette and allowed to diffuse at room temperature for 2 hours. The plates were then incubated at 37 ºC, for 24 hours. The antibacterial activity was assayed by measuring the diameter of inhibition zone around the well in millimeter [13].

Antifungal Activity: Potato dextrose agar plates were swabbed with 36 - 48 hour culture of selected fungi with sterile cotton swabs. 10 mm wells were made in each PDA plates using sterile cork borer. 100 µl of each extract were added to the wells by using micropipette and allowed to diffuse at room temperature for 2 hours. The plates were then incubated at 28 ºC, for 48 hours. The antifungal activity was assayed by measuring the diameter of the inhibition zone around the well in millimeter [16]. All the experiments were conducted in triplicates using appropriate controls.

Distilled water for aqueous extracts, ethanol and methanol for ethanolic and methanolic extracts were used as negative control for all the microbial strains.

Statistical Analysis: Data from three replicates were analysed for each experiment and analysis of variance (ANOVA) using SPSS Inc.17.0. Significant effects of treatments were determined by F-test ($P \leq 0.05$). Treatment means were separated using Tukey's HSD.

3. RESULTS AND DISCUSSION

3.1 Results

Phytochemical Analysis

The preliminary phytochemical analysis revealed the presence of flavonoids, steroids, terpenoids, saponins, phenols, tannins and anthroquinones (Table.2) in aqueous, ethanol and methanol extracts. The presence of these bioactive compounds in crude extracts is known to confer antibacterial activity against disease-causing microorganisms and offer protection of plants themselves against pathogenic microbial infections. All the selected four medicinal plants revealed positive result to preliminary phytochemical analysis. Different tests were carried out for the detection of flavonoids namely H_2SO_4 test and lead acetate test. Lead acetate test showed positive result than H_2SO_4 test and flavonoids were traced in all the extract of *C. asiaticum,* aqueous and methanol extracts of *C. martinii,* methanol extract of *C. tuberosa* but negative in *T. indica*. All the extract of *C. asiaticum*, methanol extract of *C. martini*, ethanol and methanol extract of *C. tuberosa*, and aqueous extract of *T. indica,* showed positive result to the steroids. Test for terpenoids proved that the presence of terpenoids in ethanol and methanol extracts of *C. asiaticum*, methanol extract of *C. martini*, aqueous and methanol extract of *C. tuberosa* and absent in *T. indica*. Saponions were traced only in ethanolic extract of *C. tuberosa* and absent in all other extracts. Phenols were detected by conducting two tests namely $FeCl_3$ test and lead acetate test. Phenols were present in all aqueous, methanolic and ethanolic extracts. Test for tannins proved that the presence of tannins in all the medicinal plants except *C. asiaticum*. Anthroquinones were absent in all the extracts of all the selected medicinal plants. Result obtained revealed that all the four solvent extracts of the medicinal plants gave affirmative result to the preliminary phytochemical analysis.

Table 2: Phytochemical analysis of four medicinal plants

Sl. No.	Test	*Crinum asiaticum*			*Cymbopogon martinii*			*Cyanotis tuberosa*			*Tylophora indica*		
		Aq.	Et.	Mt.	Aq.	Et.	Mt.	Aq.	Et.	Mt.	Aq.	Et.	Mt.
01	Flavonoids												
	a) H_2SO_4 test	-	-	-	-	-	-	-	-	-	-	-	-
	b) Lead acetate test	+	+	+	+	-	+	-	-	+	-	-	-
02	Steroids	+	+	+	-	-	+	-	+	+	+	-	-
03	Terpinoids	-	+	+	-	-	+	+	-	+	-	-	-
04	Saponins	-	-	-	-	-	-	-	+	-	-	-	-
05	Phenols												
	a) $FeCl_3$ test	-	+	-	-	-	-	+	-	-	+	-	-
	b) Lead acetate test.	+	+	+	+	-	+	-	-	+	-	-	-
06	Tannins	-	-	-	+	+	+	-	-	+	-	+	-
07	Anthroquinones	-	-	-	-	-	-	-	-	-	-	-	-

Note: (+) presence, (-) absence, Aq.= aqueous, Et.= ethanol, Mt.= methanol extractions

Phytochemical Analysis

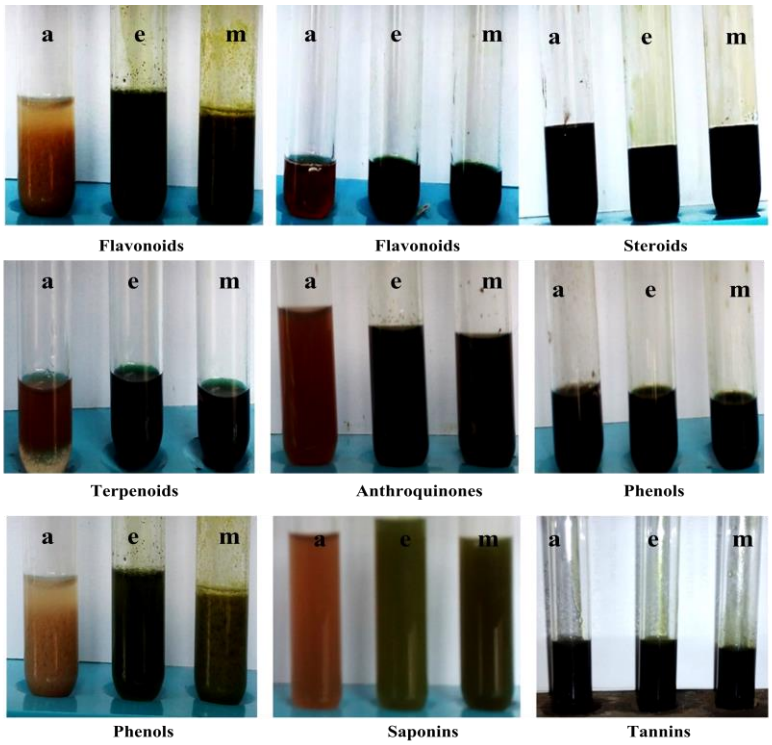

Fig 3: Phytochemical tests of *T. indica*

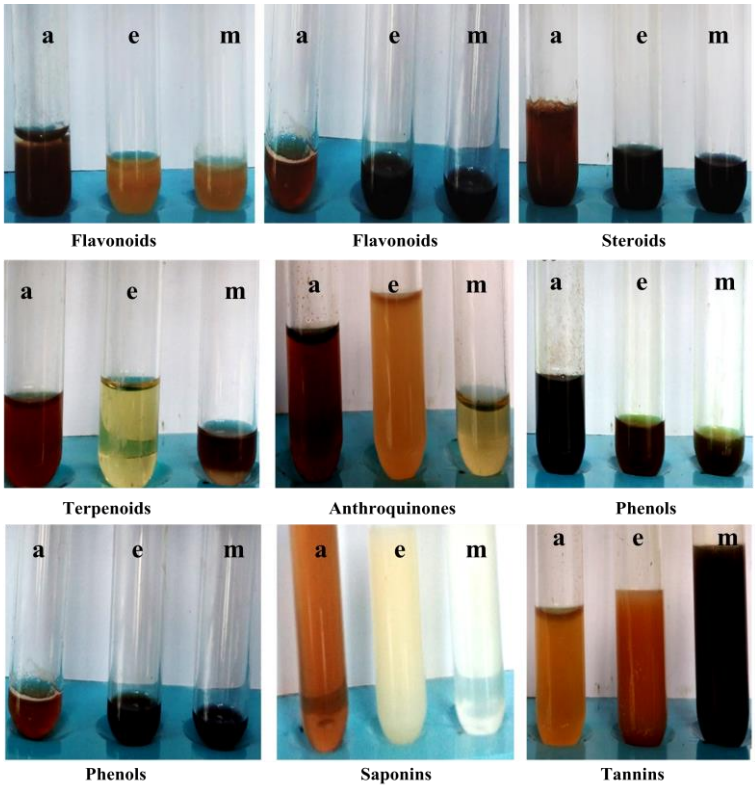

Fig 4: Phytochemical tests for *C. martini*

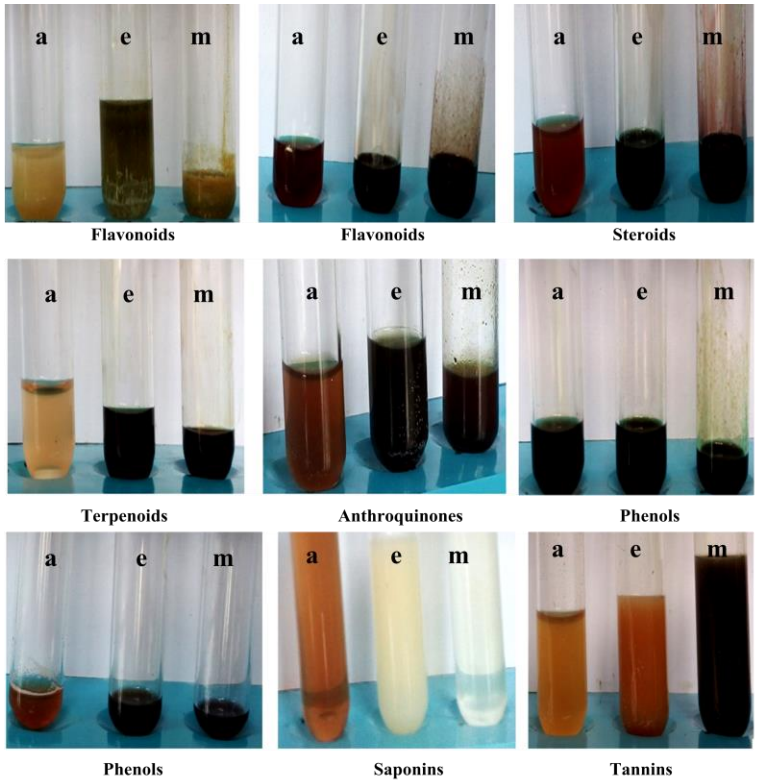

Fig 5: Phytochemical tests for *C. tuberosa*

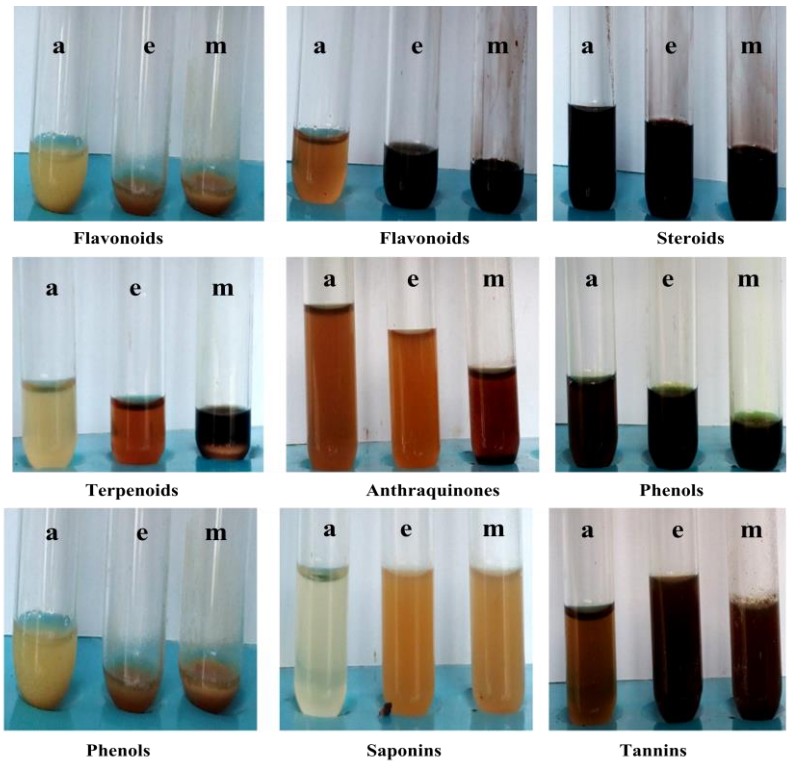

Fig 6: Phytochemical tests for *C. asiaticum*

Antimicrobial activity

In general most plant extracts of the different plant parts exhibit broad spectrum of antimicrobial activity. The antimicrobial activity of four plant species was assayed by agar well diffusion method. Four bacterial strains namely *Proteus* sp., *E. coli, S. pneumonia, S.* and *flexneri* were used as test microorganisms. Ethanol and methanol extracts from all the four plants showed highest antibacterial activity compared to aqueous extracts inhibiting bacterial growth. Among the four bacterial strains tested *Proteus* sp. was most susceptible with significant inhibition zone of (12±0.54 mm) with ethanolic extract, methanolic extracts of *C.asiaticum,* showed (6±0.16 mm) inhibition. Further 16± 0.78 mm in methanolic extracts and 6±0.11 mm inhibition was noted in ethanol extracts of *C. martinii*. Only ethanolic extracts of *C.tuberosa* expressed less inhibition (2± 0.25 mm) whereas *T. indica* represented 10±0.87mm in ethanol and 10±0.36 in methanol extracts (Table.3 and Fig.7). Extracts of *C. asiaticum* showed inhibition zone of 10±0.21 mm in methanol extract followed by 6±0.54 mm in ethanol extracts. Methanolic extracts of *C. martinii* showed 10±0.89 mm inhibition followed by 6± 0.33 mm in ethanol extract. Further 6±0.56 mm inhibition in ethanol extract of *C.tuberosa* and inhibition of 10±0.12 mm in methanol and ethanol extracts 10± 0.23 mm of *T. indica* against *E. coli* were observed (Table. 3 and Fig. 8). *S. pneumoniae* was inhibited by ethanol extract of *C. asiaticum* with an inhibition zone of (10±0.32 mm) followed by methanol (6±0.89 mm). Ethanol extract of *C. tuberosa* showed an inhibition of (10±0.14mm) followed by methanol (4±0.22 mm). Extracts of both methanol and ethanol of *T. indica* showed zone of inhibition of (6±0.35 mm) and (4±0.56 mm) respectively (Table. 3 and Fig.9). *S. flexneri* was inhibited by methanol (16±0.91

mm), ethanol (10±0.51 mm) extracts of *C. asiaticum. C. martinii* ethanol extract showed (12±0.49 mm) and (8±0.32 mm) in methanol extracts. In *C. tuberosa* ethanol extracts showed (6±0.74 mm) and methanol (10±0.13 mm) zone of inhibition whereas *T. indica* showed (6±0.65 mm) inhibition with methanol extracts only (Table.3 and Fig. 10).

Table 3: Zone of inhibition in (mm) induced by plant extracts by Agar well diffusion method

Sl. No.	Plant species	Extraction	*Proteus* sp.	*E. coli*	*Streptococcus pneumoniae*	*Shigella flexneri*
01	***Crinum asiaticum***	Aq.	-	-	-	-
		Et.	**12 ± 0.54**	6 ±0.54	10±0.32	10±0.51
		Mt.	6±0.16	10±0.21	6±0.89	**16±0.91**
02	***Cymbopogan martini***	Aq.	-	-	-	-
		Et.	6±0.11	6±0.33	-	**12±0.49**
		Mt.	**16±0.78**	10±0.89	-	8±0.32
03	***Cyanotis tuberosa***	Aq.	-	-	-	-
		Et.	2±0.25	6±0.56	10±0.14	6±0.74
		Mt.	-	-	4±0.22	10±0.13
04	***Tylophora indica***	Aq.	-	-	-	-
		Et.	10±0.87	10±0.12	6±0.35	-
		Mt.	10±0.36	10±0.23	4±0.56	6±0.65

Antibacterial activity

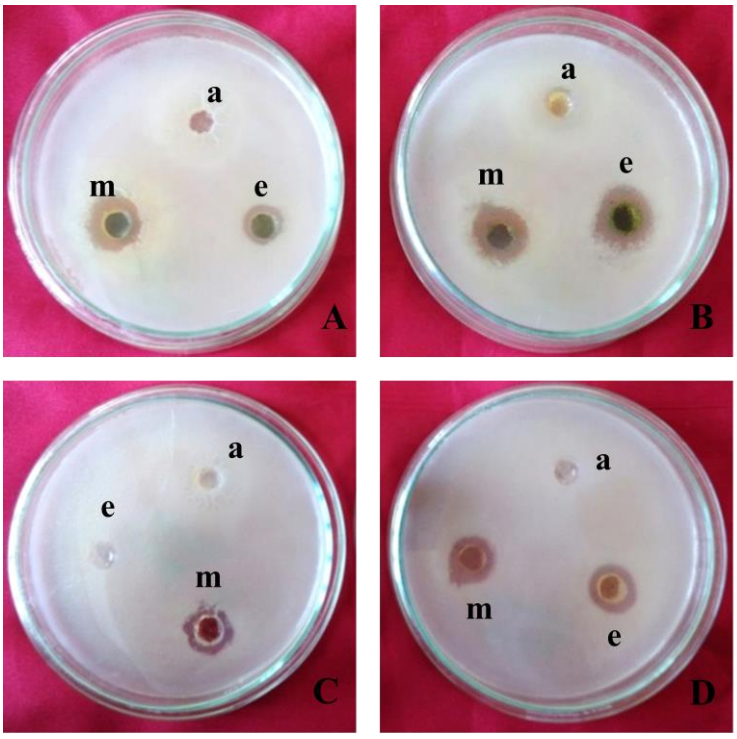

Fig 7: (A)- *C. martinii*, (B)- *T. indica*, (C)- *C. tuberosa* , (D)-*C. asiatcum* against *Proteus* sp.

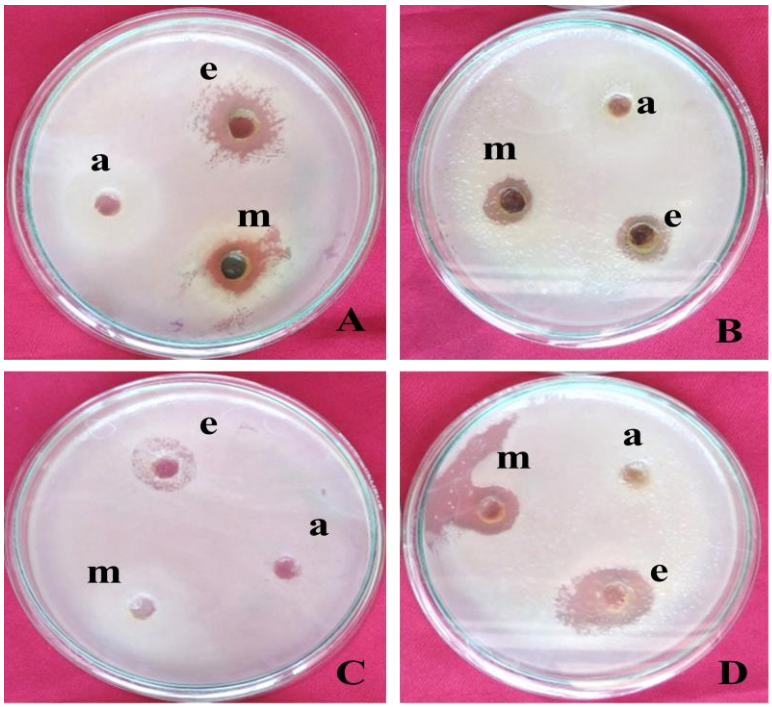

Fig 8: (A)- *C. martinii*, (B)- *T. indica*, (C)- *C. tuberosa* , (D)-*C. asiatcum* against *E. coli*

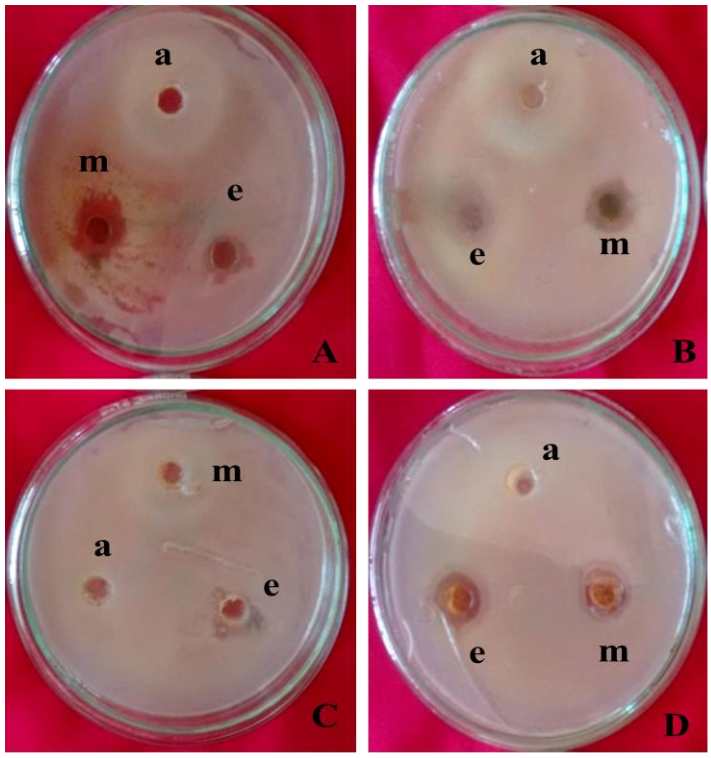

Fig 9: (A)- *C. martinii*, (B)- *T. indica*, (C)- *C. tuberosa* , (D)-*C. asiatcum* against *S. pneumoniae*

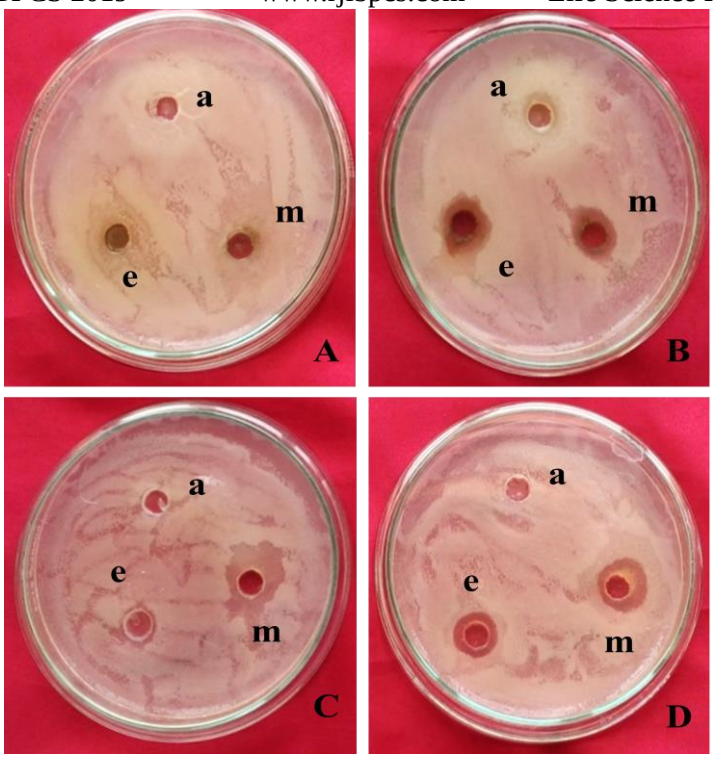

Fig 10: (A)- *C. martinii*, (B)- *T. indica*, (C)- *C. tuberosa* , (D)-*C. asiaticum* against *S. flexneri*.

Antifungal activity

Antifungal activity was carried out by agar well diffusion method against two fungal sps. *T. viride* and *Cladosporium* sp. and there was no significant activity exhibited by both aqueous and ethanolic and methanolic extracts of plants.

Table 4 : Zone of inhibition in (mm) induced by plant extracts by agar well diffusion method

Sl. No.	Plant species	Extraction	*T.viride*	*Cladosporium* sp.
1	*C. asiaticum*	Aq.	-	-
		Et.	12±0.87	7±0.14
		Mt.	4±0.22	9±0.63
2	*C. martinii*	Aq.	-	-
		Et.	7±0.11	4±0.33
		Mt.	8±0.27	8±0.21
3	*C. tuberosa*	Aq.	-	-
		Et.	-	4±0.61
		Mt.	-	-
4	*T. indica*	Aq.	-	-
		Et.	7±0.54	-
		Mt.	8±0.01	-

Note: (+) presence, (-) absence, Aq.= aqueous, Et.= ethanol, Mt.= methanol extractions

Antifungal activity

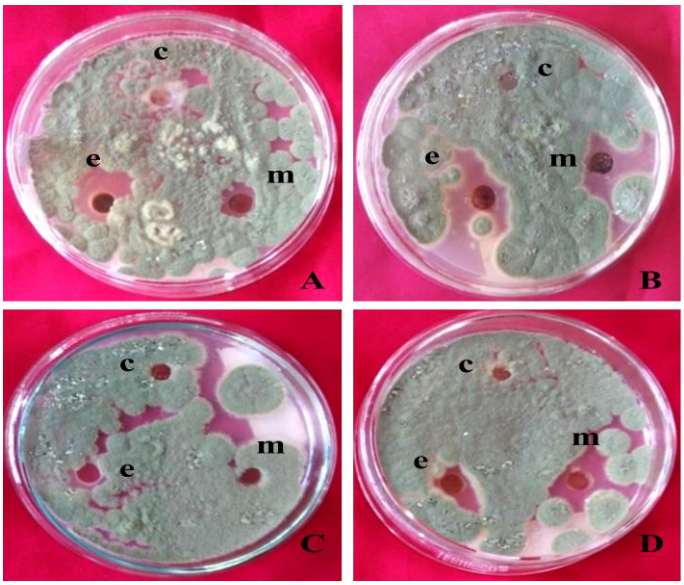

Fig 11: (A)- *C. martinii*, (B)-*T. indica*, (C)- *C. tuberosa*, (D) - *C. asiaticum* against *T. viride*

Methanol and ethanol extracts *C. asiaticum* showed (4±0.22 mm) and (12±0.87 mm) of inhibition against *T. viride* respectively. Ethanol extract of *C.martinii* showed 7±0.11mm and methanol extract 8±0.27 mm inhibtion. Further *T. indica* showed inhibition of 8±0.01 mm in methanol extract and 7±0.54 mm in ethanolic extract whereas inhibition was nil in the all extracts of *C. tuberosa* (Table.4 and Fig.11). About 9±0.54 mm inhbition in methanol and 7±0.14mm in ethanolic extracts of *C. asiaticum* were observed against *Cladosporium* sp. Methanol and ethanol extracts of *C.martinii* (8±0.21mm) and (4±0.33 mm) inhibition was observed. *C. tuberosa* ethanol extract showed 4± 0.61 mm inhibition and there was no inhibition against the test fungi. *T. indica* did not show any inhibition in both extracts (Table. 4 and Fig. 12).

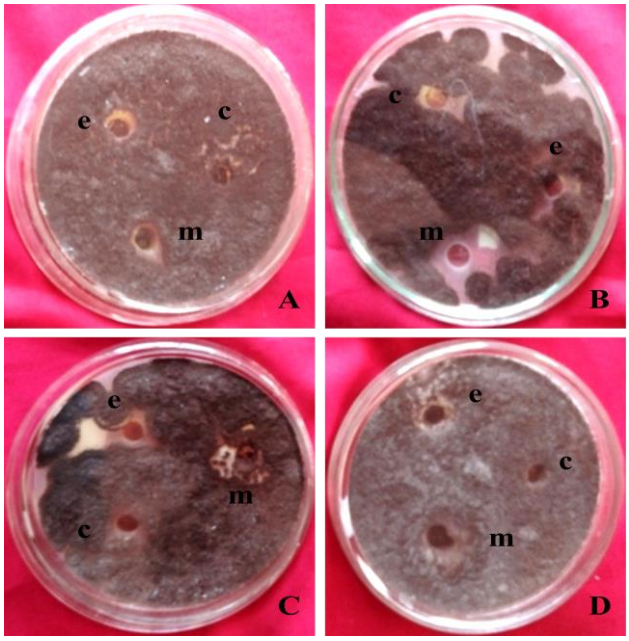

Fig. 12: (A)- *C. martinii*, (B)-*T. indica*, (C)- *C. tuberosa*, (D) - *C. asiaticum* against *Cladosporium* sp.

3.2 DISCUSSION

The uses of antibiotics are widespread in clinical medicine, agriculture, and veterinary promote the development of antibiotic resistances among infectious microbial strains and eventually reflects a very serious problem in the treatment of pathogenic microbes [17]. This has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the above disadvantages [18]. Natural products are typically secondary metabolites, produced by plants and microorganisms in response to external stimuli such as nutritional changes. They are widely used in the pharmaceutical industry for their remarkable structural diversity and range of pharmacological activities [19]. Analysis of past, present and future of medicinal plants, both as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infection agents [20]. Phytochemicals have time-honored an increasing attention because of interesting new discoveries considering their biological activities [21]. Analysis and antimicrobial study of medicinal plants is a very significant way to establish that the plant species may be used as potent drugs. The high cost and side effects of synthesized drugs are forcing the scientists to research alternative sources for the treatment of diseases. saponins, terpenes, glycosides, essential oils and polyphenols are a very promising drug discovery. In this study, it was found that the presence of tannins, saponins, flavonoids alkaloids, terpenoids and anthroquinones and can be used as an important source of phytochemical and antimicrobial activity. On the basis of this antimicrobial study, it is clear that the selected plant shows a considerable activity against selected microbial strains. Therefore, this study indicates that these plants may be used as potent antibacterial drugs of natural origin. A further work may emphasize the isolation and characterization of effective compounds. Extraction of phytochemicals [22] phytochemical analysis are in support with the works [23-24]. The obtained results of the current study are in parallel with the results [25-26] where methanolic extracts of *C. asiaticum* against *S. flexneri* (16± 0.91mm) and (12± 0.54mm) inhibition of *Proteus* sp with ethanol extracts. The antimicrobial activity and chemical constituents of plants were confirmed in different studies [27-28]. Ethanol extracts of *C. asiaticum* showed inhibition zone of (12± 0.87mm) against *T.viride* and (9± 0.63 mm) inhibition against *Cladosporium* sp. which are similar to the work [29], antimicrobial activity of crude ethanolic extracts plants used in traditional medicine against five species of microorganisms [30-32]. Most of the extracts had substantial inhibitory abilities on the growth of the tested cultures. The different rates of inhibition could probably be due to the quantity of the phytochemical compounds present in the extracts. This can be owing to the antagonist activity of the various phytochemical that may be present in the extract and such a plant may not offer alternative medicare against such disease caused by the organisms.

4. CONCLUSION

With the aforementioned affirmative results from *in vitro* test with microorganisms, there still remains jeopardy in our hearts concerning the possible toxicity potentials of those plants. Scientists from divergent fields are investigating plants anew with an eye to their antimicrobial usefulness. A sagacity of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro*. All these compounds should be subjected to animal and human studies to determine their effectiveness in whole-organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of results would be assisted. Also, substitutes in mechanisms of infection, prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened for currently. Attention to these issues could escort in a badly needed new era of chemotherapeutic treatment of infection by using plant-derived principles. The findings of the study may be helpful to the future investigators on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds. Moreover, research in this area should be carried on until the agent accountable for the activity has been determined or, as the case may be, the most active fraction or extracts have been discovered. Finally, diverse studies on the mechanisms of action, interactions with antibiotics or other medicinal plants or compounds, and the pharmacokinetic profile of the extracts should be given high priority.

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CONFLICT OF INTEREST

Authors have declared that no competing interests exist.

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