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Research Article

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS, QUANTITATIVE ANALYSIS AND ANTIMICROBIAL EFFICACY OF POTENTIAL HERBS AGAINST SELECTED MICROBES

RAJESWARI A*

Department of Microbiology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India. Email: raji.anburaj@gmail.com

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ABSTRACT

Objectives: The aim was to evaluate the physicochemical composition, phytochemical analysis and antimicrobial activity of plant extracts.

Methods: The plant samples (*Citrus reticulata*, *Camellia sinensis* and *Punica granatum*) were extracted using various solvents (acetone, butanol, chloroform, hexane, methanol and water) and antimicrobial assay was performed against selected microbes *viz.*, *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeuroginosa, Salmonella typhi, Staphylococcus aureus, Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus, Fusarium oxysporum* and *Fusarium verticilloides.*

Results: Acetone and aqueous extracts (23 mm) of *C. sinensis* and *P. granatum* acquired maximum inhibition. *P. granatum* found to possess strong antifungal activity in acetone and methanol extract (19 mm) against *F. verticilloides*.

Conclusion: These findings suggest that *C. sinensis* and *P. granatum* act as a potential source of the antimicrobial agent.

Keywords: Phytonutrients, Plant extracts, Antimicrobial screening, Agar-well dilution, Inhibition.

INTRODUCTION

In recent years, multiple drug/chemical resistance in both human and plant pathogenic organisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases [1]. Plants have been shown to be good alternatives to synthetic chemical antimicrobial agents and antibiotics, antimicrobial resistance and the emergence of previously uncommon infections that have been reported to be on the increase due to inappropriate or widespread overuse of antimicrobials [2,3]. Phytochemical studies have attracted the attention of plant scientists due to the development of innovative techniques. These techniques played a significant role in the search for additional resources of raw material for the pharmaceutical industry (phytochemicals) [4]. These phytochemicals, often secondary metabolites present in smaller quantities in higher plants, include the alkaloids, flavonoids, saponin, phenols, terpenoids, tannins and many others [5].

The resistance of microbes and appearance of strains with reduced susceptibility to antibiotics are continuously increasing due to the indiscriminate use of commercial antibacterial treatment of infectious disease [6]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics and are high major burning global issues in treating infectious diseases [7]. Therefore, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [8-10]. Natural products are directly used as therapeutic agents as well as starting material for the synthesis of synthetic drugs or as models for pharmacologically active compounds [11]. There is currently enormous surge of significance in the utilization, progress and preservation of the medicinal plants throughout the world. The presence of these phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drug for human use [12]. Therefore, this study aims to evaluate the phytoconstituents and potential of plant extract against pathogens.

METHODS

Plant materials

The plant materials such as *Citrus reticulata, Camellia sinensis* and *Punica granatum* were chosen for the study. The leaves of *C. sinensis* were collected from Idukki region, Kerala, India. The rind parts of *C. reticulata* and *P. grantum* were collected from in and around Madurai region, Tamil Nadu, India. The plant materials were washed thoroughly with tap water, shade dried and homogenized to a fine powder and stored in air tight containers.

Physicochemical parameters

The physicochemical parameters like pH, total ash value, moisture content, alcohol soluble extractive value (ASEV) and water soluble extractive value (WSEV) were determined as per WHO guidelines [13].

Phytochemical assessment of plants

The crude powder of the plant samples was extracted using different solvents and subjected to qualitative phytochemical analysis [14].

Quantitative analysis

Quantitative analysis of plant samples was done to determine the amount of carbohydrate, fat, protein, crude fiber, sugars (total sugar and reducing sugar) and vitamin C [15]. Phytoconstituents such as alkaloid, total phenol, saponin, flavonoid, tannin, condensed tannin, riboflavin and thiamine [16] and phenolic compounds [17] were analyzed by thin-layer chromatography (TLC).

Microorganisms

The microbial cultures of *Escherichia coli* 433, *Pseudomonas aeuroginosa 1934*, *Staphylococcus aureus* 1473, *K. pneumonia* 432, *Salmonella typhi* 733, *Aspergillus niger* 10130, *Aspergillus flavus* 9064, *Aspergillus parasiticus* 6777, *Fusarium oxysporum* 4356, *Fusarium verticilloides* 3322 were procured from the microbial type culture collection, Institute of Microbial Technology, Chandigarh, India. The bacterial isolates were maintained on nutrient agar slants at 4°C and the fungal cultures were maintained on potato dextrose broth at 25°C.

Preparation of inoculum
The bacterial cultures were inoculated into nutrient broth and incubated for 24 hrs at 37°C. The growth was compared with 0.5 McFarland; the turbidity of the medium indicates the growth of organisms, while the fungal cultures were inoculated into potato dextrose broth and allowed to incubate at 25°C for 48 hrs [18].

Antimicrobial screening
The agar well diffusion method was employed for the determination of antimicrobial activity of the extracts [19]. The test organism such as bacteria and fungi were respectively lawn cultured on nutrient agar and rose Bengal agar by using sterile cotton swabs. The wells (6 mm in diameter) were cut from the agar plates using a cork borer. 60 µl of the extracts (16 mg/ml) were poured into the well using a sterile micropipette. The plates were incubated at 37°C for 24 hrs for bacteria and 25°C at 48 hrs for fungi. After the incubation, the zone inhibition was measured by the standard scale (Hi-media) in millimeter.

RESULTS

Physicochemical analysis
Physicochemical analysis of plant samples revealed the presence of moisture, ash content, pH, ASEV and WSEV. The results were illustrated in Table 1. *C. sinensis* acquired higher pH 6.7, followed by *C. reticulata* (5.2) and *P. granatum* (4.7). *C. reticulata* (88.4%) possess maximum moisture content, whereas was lower in case of *C. sinensis* (6.1%). Leaves of *C. sinensis* (5.9%) accumulate more ash content, whereas found to be less in peel parts of *P. granatum* (2%). Alcohol (22.2%) and water (17.6%) soluble extractive value was found to be higher in *Coffea arabica*.

Phytochemical assessment of plants
The results of phytochemical analysis of plant samples were presented in Table 2. Analysis of four different solvent extracts of the plants demonstrated the presence of phytochemicals such as alkaloids, flavonoids, glycosides, steroids, tannin, saponin, protein and carbohydrate. Flavonoids specified positive result for the water extracts of *C. reticulata*, *C. sinensis* and *P. granatum*. Apart from the hexane extract, all the solvent possess glycoside content in *C. reticulata* and *P. granatum*. *C. sinensis* exhibited positive result towards glycosides, tannin, protein and carbohydrate. Except alkaloids, water and methanol extracts of *C. reticulata* possess majority of phytochemical constituents. Acetone, methanol and water extracts of *P. granatum* confirmed positive result for the test. Steroid displayed positive result toward methanol extract. Saponin was absent in the hexane extract of all plant samples. Chloroform and hexane extract produced a negative result to steroid, tannin, saponin, protein, and carbohydrate.

Quantitative analysis
The results for quantitative analysis of the plant samples were illustrated in Table 3. *C. sinensis* found to possess the maximum amount of amino acid (37.7 mg/ml), fiber (9.5 mg/ml) and reducing sugars (16.4 mg/ml). Carbohydrate content was found to be higher in *C. reticulata* (21.1 mg/ml), *P. granatum* (14.5 mg/ml) and *C. sinensis* (0.2 mg/ml). Significant amount of vitamin C existed in *C. reticulata* (5.1 mg/ml) followed by *P. granatum* (1.9 mg/ml), *C. sinensis* (1.5 mg/ml). Flavonoid content (81.3 mg/ml) was found to be higher in *P. granatum* (81.3 mg/ml) whereas lower in *C. sinensis* (19.7 mg/ml) and *C. reticulata* (0.07 mg/ml). Substantial amount of phenol content were present in *C. sinensis* (62.3 mg/ml), *P. granatum* (38.9 mg/ml), and *C. reticulata* (18.1 mg/ml). The quantity of alkaloid (2.8 mg/ml), tannin (23.l mg/ml) and condensed tannin were found to be higher in *P. granatum* (12.2 mg/ml), while lower in *C. reticulata* (tannin - 0.45 mg/ml). *P. granatum* (5 mg/ml) possess maximum saponin content, whereas trace amount were present in *C. sinensis*. Riboflavin and thiamine were present in *C. reticulata* (0.1 mg/ml), whereas negligible in *C. sinensis*. Samples were evaluated for the presence of phenolic compounds, such as gallic acid. Gallic acid possess maximum R_f value, followed by *P. granatum* (0.88 mg/ml), *C. sinensis* (0.86 mg/ml) and *C. reticulata* (0.69 mg/ml). Gallic acid was used as the standard for determination of compounds (0.82 mg/ml).

Evaluation of antibacterial activity
In vitro preliminary screening of the antibacterial activity of the plant extracts was represented in Fig. 1a-e. The maximum inhibitory effect was recorded in methanol (14 mm) and aqueous (19 mm) extracts of *C. reticulata* against *P. aeuroginosa*, followed by acetone extract against *S. aureus* (11 mm). Butanol extracts against *S. typhi* (9 mm) had acquired maximum inhibition, when compared with *Klebsiella pneumoniae* and *S. typhi* (6 mm), whereas no significant effect was observed against

Table 1: Physicochemical characteristics of plant samples

P	pH	M	TA	ASEV (%)	WSEV (%)
C. reticulata	5.2	88.4±0.3	5.60±0.09	22.2	17.6
C. sinensis	6.7	6.13±0.03	5.91±0.06	15.5	12.4
P. granatum	4.7	60.3±0.4	2.00±0.1	18.12	12.9

P: Plant samples, C. reticulata: Citrus reticulata, C. sinensis: Camellia sinensis, P. granatum: Punica granatum, M: moisture, ASEV: Alcohol-soluble extractive value, WSEV: Water-soluble extractive value. Moisture and total ash values were represented as mean±SD. SD: Standard deviation

Table 2: Qualitative phytochemical screening of plant samples using different solvents

P	*C. reticulata*						*C. sinensis*						*P. granatum*					
	A	B	C	H	M	W	A	B	C	H	M	W	A	B	C	H	M	W
AL	+	−	+	+	+	−	−	+	+	+	+	−	+	−	+	−	+	+
F	−	−	+	−	+	+	+	−	−	−	−	+	+	+	−	−	+	+
G	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+
S	+	−	+	−	+	+	+	−	−	−	+	−	+	−	−	−	+	+
T	+	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	+	+
S	+	−	+	−	+	−	+	−	−	−	+	+	+	−	−	−	+	+
P	+	−	−	−	+	+	+	+	+	+	+	+	+	+	−	−	+	+
C	+	+	−	−	+	+	+	+	+	+	+	+	+	+	−	−	+	+

P: Parameters, AL: Alkaloid, F: Flavonoid, G: Glycosides, S: Steroid, T: Tannin, S: Saponin, P: Protein, C: Carbohydrate, Solvents - A: Acetone, B: Butanol, C: Chloroform, H: Hexane, M: Methanol, W: Water, +: Present, −: Absent, *C. reticulata: Citrus reticulata, C. sinensis: Camellia sinensis, P. granatum: Punica granatum*

Table 3: Quantitative analysis of phytochemical constituents of plant samples

PM	Plant samples used in the study (mg/ml)		
	C. reticulata	*C. sinensis*	*P. granatum*
AA	1.7±0.3	37.7±4.6	Tr
C	21.1±0.05	0.21±0.3	14.5±0.06
F	Tr	0.15±0.06	1.02±0.04
P	9.56±0.1	2.2±0.1	2.59±0.1
FI	6.50±0.1	9.56±0.4	3.96±0.04
TS	8.24±0.7	3.25±0.1	12.6±0.08
RS	6.11±0.01	16.4±2.3	4.8±1.5
V	5.1±1.7	1.5±0.20	1.9±0.06
A	1.20±0.2	0.42±0.4	2.85±0.05
FL	0.07±0.1	19.17±0.2	81.33±6.1
S	2.56±0.02	Tr	5.0±0.11
T	0.45±0.2	26.4±3.1	23.1±0.1
CT	0.52±0.8	0.42±0.09	12.2±0.02
TP	18.1±0.1	62.3±0.6	38.9±6.4
RI	0.04±0.09	0.02±0.6	0.1±0.3
Th	0.1±0.02	Tr	0.09±0.4
Rf	0.72±0.2	0.86±0.2	0.88±0.1

C. reticulata: Citrus reticulata, C. sinensis: Camellia sinensis, P. granatum: Punica granatum, PM: Parameters, A.A: Amino acids, C: Carbohydrate, F: Fat, P: Protein, FI: Fibre, TS: Total sugar, RS: Reducing sugars, V: Vitamin C, A: Alkaloid, FL: Flavonoid, S: Saponin, T: Tannin, CT: Condensed tannin, TP: Total phenol, R: Riboflavin, Th: Thiamine, Tr: Trace amounts, Rf: Retention factor values of phenolic compounds, Values are represented as mean±SD. SD: Standard deviation

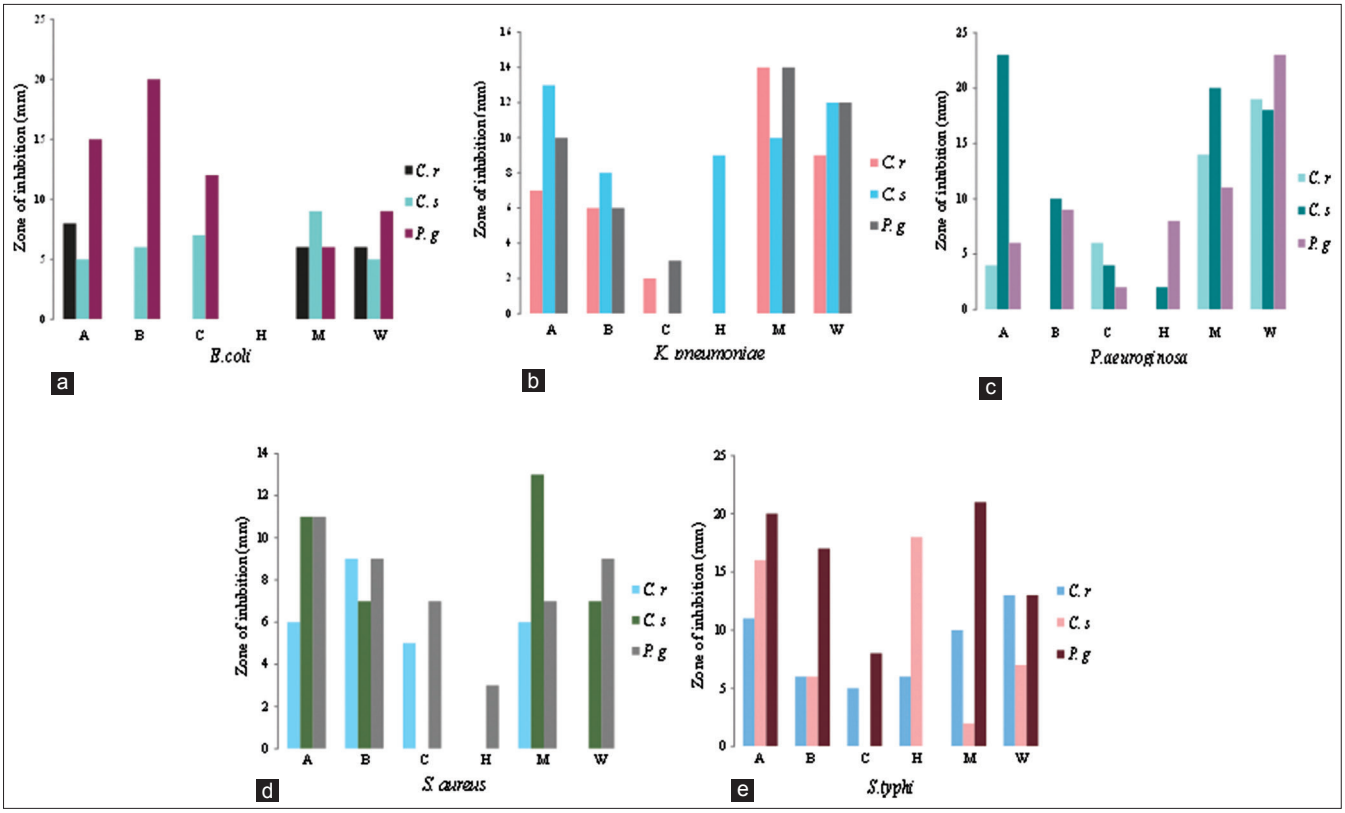

Fig. 1: Antibacterial assay of plant extract against various pathogens, *C.r: Citrus reticulata; C.s: Camellia sinensis; P.g: Punica granatum*, A: Acetone, B: Butanol, C: Chloroform, H: Hexane, M: Methanol, W: Water, (a) *Escherichia coli*, (b) *Klebsiella pneumoniae*, (c) *Pseudomonas aeuroginosa*, (d) *Staphylococcus aureus*, (e) *Salmonella typhi*

E. coli and *P. aeuroginosa*. Except *S. aureus* (6 mm) all the organisms were resistant towards the hexane extracts. Insignificant effect was found toward the chloroform extract against *E. coli*, while *P. aeuroginosa* remained sensitive having an inhibition of about 6 mm.

The strongest inhibition was recorded in the acetone extracts of *C. sinensis* against *P. aeuroginosa* (23 mm), followed by *S. aureus* (16 mm), *K. pneumoniae* (13 mm), *S. typhi* (11 mm), whereas *E. coli* (5 mm) was considered as the resistant strain having very less inhibition, moderate inhibition was denoted in the butanol extracts against *P. aeuroginosa* (10 mm) and the zone of diameter was very low against *S. aureus* and *E. coli* (6 mm). Minimum inhibition was found in the chloroform extracts against *E. coli* (7 mm), while *K. pneumoniae*, *S. typhi* and *S. aureus* were considered as the resistant strains. *S. aureus* was considered as sensitive strain against hexane extracts (18 mm) and no significant effect was observed against *E. coli* and *S. typhi*. Methanol and water extracts of *C. sinensis* (20 mm, 18 mm) produced very good inhibitory effect, whereas water extract against *S. aureus* (10 mm) was found to be resistant, followed by *S. typhi* (7 mm) in aqueous extracts.

Aqueous extract (23 mm) of *P. granatum* exhibited superior activity against *P. aeuroginosa,* followed by methanol (21 mm) and acetone (20 mm) extracts of *S. aureus.* Consequently, butanol extracts against *E. coli* (20 mm) showed maximum inhibition followed by *S. aureus* (17 mm). *E. coli* and *K. pneumoniae* remained resistant toward the hexane extract, having no inhibition. *S. aureus*, *K. pneumoniae* and *S. typhi* were found to be sensitive against methanol (21 mm, 14 mm) and acetone (11 mm), respectively. Chloroform (2 mm) extracts indicated to have least inhibition against *P. aeuroginosa*, whereas *E. coli* produced moderate inhibition against chloroform extract (12 mm).

Evaluation of antifungal activity

In vitro preliminary screening of the antifungal activity of the plant extracts was represented in Fig. 2a-e. *A. niger* (13 mm) and *A. parasiticus* (12 mm) remained sensitive toward methanolic extracts of *C. reticulata* while hexane (2 mm) and acetone (5 mm) extract displayed minimum inhibition. Butanol extract (10 mm) against *A. flavus* exhibited modest inhibition. *F. verticilloides* were sensitive toward hexane extract (14 mm) having higher inhibition while compared to the chloroform extract (3 mm).

Against the selected fungal strains, *C. sinensis* reported to have minimum inhibitory effect. The maximum zone of inhibition was observed in butanol (8 mm) extract and aqueous (3 mm) extract against *A. flavus*. Subsequently, *F. oxysporum* and *F. verticilloides* produced an inhibition of 7 mm, whereas insignificant effect was obtained in an aqueous extract. *A. parasiticus* was found to be sensitive against the butanol extracts (9 mm) of tea and resistant towards hexane and water extracts. In chloroform extract, the diameter of the zone recorded was about (6 mm), whereas least inhibition was noted in acetone and water extracts (3 mm).

P. granatum possess strong anti-fungal activity against *F. verticilloides* in acetone and methanol extracts showing an inhibition of about (19 mm). Subsequently, acetone extract (16 mm) were effective against *A. parasiticus*, whereas least inhibition was found in an aqueous extract (1 mm) of *P. granatum*. The fungal strains *A. flavus* and *F. oxysporum* was found to be sensitive against butanol (15 mm) and methanol (13 mm) extracts. Butanol extract produced an inhibition of about 11 mm against *A. niger*, whereas resistant to chloroform extract.

DISCUSSION

Plants are important sources of potentially useful substances for the development of new therapeutic agents. Various phytochemical compounds as secondary metabolites have been implicated in plants as the conferment of antibacterial activities [20,21]. Moisture content of drugs could be at a minimal level to discourage the growth of bacteria,

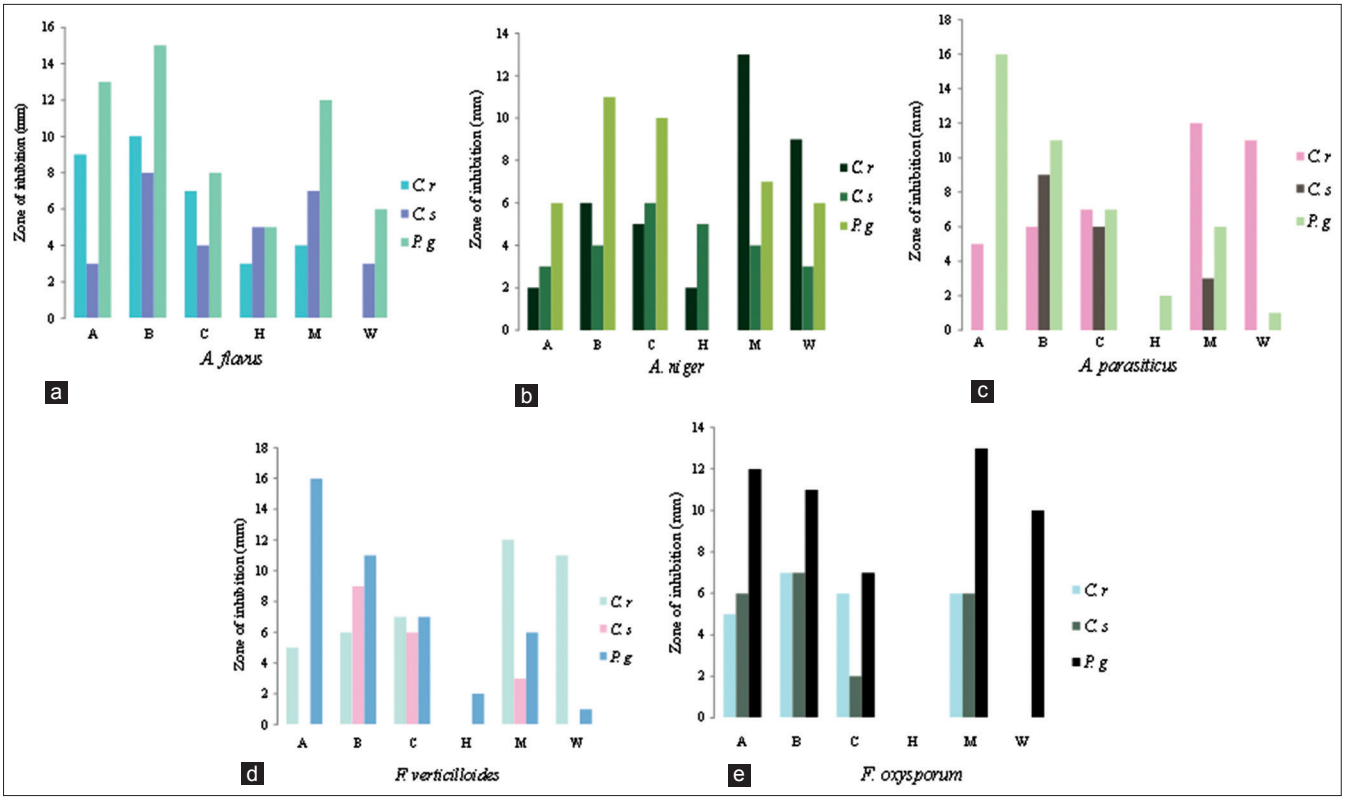

Fig. 2: Antifungal assay of plant extract against various pathogens, *C.r: Citrus reticulata, C.s: Camellia sinensis, P.g: Punica granatum,* A: Acetone, B: Butanol, C: Chloroform, H: Hexane, M: Methanol, W: Water, (a) *Aspergillus flavus,* (b) *Aspergillus niger*, (c) *Aspergillus parasiticus*, (d) *Fusarium oxysporum,* (e) *Fusarium verticilloides*

yeast or fungi during storage. Ash values are used to determine quality and purity of crude drug. It indicates the presence of various impurities such as carbonate, oxalate, and silicate. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in the estimation of specific constituents soluble in a particular solvent [22]. Acid insoluble ash measures the amount of silica present, especially sand. Water soluble ash is the water soluble portion of the total ash [23,24]. Ash value of the different tea brands ranged from 5.52 to 9.28%, which gives the rough idea of the mineral content in the samples. A literature revealed that the WSEV in tea ranges from 8.86 to 12.97%, indicating the presence of sugar, acids and inorganic compounds, while ASEV was 4.22-7.05%, which also indicted the presence of polar constituents [25]. Percentage of ash (5.91%) and water soluble (12.4%) extractive value in *C. sinensis* corroborated with the results of the previous investigation. Present examinations revealed that maximum moisture content has been reported in *C. reticulata* (88%), when compared to *P. granatum* (60%) and *C. sinensis* (6%).

Chemical structure of *C. sinensis* is mainly composed of polyphenolic compounds, such as catechins and flavonoids, alkaloids with special emphasis on caffeine and theophylline, volatile oils, polysaccharides, amino acids, lipids, vitamins, inorganic elements such as aluminium, fluorine and manganese [26]. *Citrus limon* and *C. sinensis* peels have a high quantity of saponin, which has hemolytic activity and cholesterol binding properties. Present examination implies that *P. granatum* (5 mg/ml) possess maximum saponin content followed by *C. reticulata* (2.5 mg/ml). Therefore, in addition to their use as drugs, citrus peels can be used as a food preservative or even as a food supplement as they are highly nutritive [27]. Polyphenols are a main group of pomegranate phytochemicals, which act as phytochemical antioxidants with potential health related benefits [28]. Condensed tannins are also known as proanthocyanidins, which are polymeric flavonoid molecules that are found in a range of higher plant species [29]. Recent studies reveal the fact that there were significant differences in the condensed tannin (12.14-12.5 mg catechin/100 g) and the total sugar level of pomegranate content (16.8-22.7 g/100 g) [30]. Current research indicates that *C. sinensis* (26.4 mg/ml) and *P. granatum* (23.1 mg/ml) possess higher tannin content, while condensed tannin was fairly distributed in *P. granatum* (12.2 mg/ml). Thereby, the results indicate that the amount of condensed tannin in *P. granatum* correlates with former reports.

Green tea possesses antibacterial activity due to the presence of polyphenols, specific antioxidant polyphenols called catechins play an important role [31]. Earlier studies denoted that antibacterial activity of the ethanol and aqueous extract of *C. sinensis* leaf against the test organisms taken in their study (*E. coli*: Aqueous extract - 7 mm, ethanol extract - 3 mm; *K. pneumoniae*: 3 mm for both the extracts*; S. aureus*: Aqueous extract - 1 mm, ethanol extract - 2 mm) [25]. Peel of lemon reported to be an astringent and a good antimicrobial agent. This is an important finding as certain skin flora like *Pseudomonas* and *Micrococcus* can grow in the presence of sebum, especially when it is secreted in excess, can cause purulent skin infections [32]. Earlier studies denoted that antibacterial activity of the ethanol and aqueous extract of *C. sinensis* leaf against the test organisms taken in their study (*E. coli*: Aqueous extract - 7 mm, ethanol extract - 3 mm; *K. pneumoniae*: 3 mm for both the extracts*; S. aureus*: Aqueous extract - 1 mm, ethanol extract - 2 mm) [33]. Methanolic extract of orange peels consist of a good antioxidant and antibacterial activity as compared to hydromethanolic extract [34]. According to a previous report the antibacterial activity of pomegranate is due to the presence of tannins such as ellagitannins and flavonoids [35]. The present work signified that the aqueous extract (23 mm) of *P. granatum* exhibited superior activity against *P. aeuroginosa,* followed by methanol (21 mm) and acetone (20 mm) extracts of *S. aureus*. Consequently, butanol extracts against *E. coli* (20 mm) showed maximum inhibition followed by *S. aureus* (17 mm).

Preceding studies reported that oil obtained from *C. reticulata* peel acquired a maximum inhibition of 30 mm against *Candida albicans* [27]. Contemporary work implies that *A. niger* (13 mm) and *A. parasiticus* (12 mm) produced moderate inhibition against methanolic extracts of *C. reticulata*, thereby results signified that inhibitory activity was less against the selected fungal strains. The antimicrobial activity of *P. granatum* peel extract might be related to the action of its antibiotic compounds or to the presence of metabolic toxins. *P. granatum* and *Syzygium cumini* extracts exerted strong antifungal activity and can be a source for the development of new therapeutic agents, as they inhibited the growth of *Candida* [36]. The disc diffusion assay showed that the fruit extracts have different degrees of bacterial and fungal growth inhibition, depending on the strains [37]. Phytochemicals are non-nutritive plant chemicals that have disease preventive properties [38]. Recent findings reported that maximum inhibition in rind extract of pomegranate (23 mm), followed by juice extract (20 mm), the least antifungal effect was recorded by seed extract (8 mm) [39]. Present results slightly correlated with prior reports as the rind part of *P. granatum* possess strong anti-fungal activity against *F. verticilloides* in acetone and methanol extracts showing an inhibition of about 19 mm. Subsequently, acetone extract (16 mm) showed very good inhibition against *A. parasiticus*, whereas moderate inhibition was found in aqueous extract of *P. granatum*, *A. flavus* and *F. oxysporum* was found to be sensitive against butanol (15 mm) and methanol (13 mm) extracts.

The plants were assessed for the presence of phenolic compounds *P. granatum* (0.8 mg/ml) constitutes maximum R_f value, followed by *C. reticulata* (0.69 mg/ml). Gallic acid was used as the standard for determination of phenolic compounds (0.82 mg/ml). The quality control and quality assurance still remains a challenge because of the high variability of chemical components involved. Due to natural variability, chemical analysis of plant material is a great challenge and requires special approaches. TLC continues to be an important method for qualitative analysis of plant products because of its inherent advantages many samples can be analyzed simultaneously, multiple separation techniques and detection procedures can be applied [40]. Gallic acid is also used to manufacture propyl gallate, an antioxidant used in the food industry. Moreover to produce pyrogallol, this is used in staining fur, leather and hair, and also as photographic developer. Based on this inquiry, plants can be used for the large scale production of gallic acid, as the preferred plant samples are rich in tannin, tannin acyl hydrolase catalyzes the ester bonds by producing gallic acid and glucose [41].

Present investigations provide information in respect of their physicochemical analysis, phytochemical assessment, nutritional composition and antimicrobial properties of plant samples. Therefore, existing work signified the fact that *P. granatum* possess a broad spectrum of antimicrobial activity.

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