Phytochemical investigation andAntimicrobial potential of a novel Polyherbal formulation

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ABSTRACT

In ayurveda, herbal formulations have been used for its medicinal and therapeutic applications since ancient days. Plants are rich source of phytochemicals and have been explored for treatment of various diseases. In the present investigation, antimicrobial evaluation of a novel polyherbal formulation(PHF) containing of Trigonella foenum graecum, Cinnamomom verum, Aquilaria malaccensis, Azadirachta indica, Nigella sativa and Carthamus tinctorious was done. Phytochemical investigation revealed the presence of alkaloids, flavonoids, saponins, tannins, and glycosides. For antibacterial study, ofloxacin was used as standard drug; gram positive bateria- Bacillus subtilis, Shigella dysenteria and Staphyllococcus aureus and gram negative bacteria-Psuedomonas aeroginosa and Escherichia coli were used. For antifungal activity, ketoconazole was used as standard drug; Candida albicans and Aspergillus niger were used. The ethanolic extract of the PHF demonstrated better antimicrobial activity than the petroleum ether extract. The zone of inhibition was strong for 100µg/ml ethanolic extract against gram positive, gram negative and fungi strains. The PHEE3 showed significant activity of 21±0.421 to 26.83±0.404mm, then by PHEE3 ranging between 20.5±0.763 to 26.1±0.477 mm against all strains of microbes used suggesting synergistic action by the plants used. Presence of phytochemicals like tannins, flavonoids and other hydroxylated polyphenols, formed as secondary metabolites may be responsible for the antimicrobial activity of the PHF.

Keywords: ayurveda, polyherbal formulation, phytochemical investigation, antibacterial, antifungal.

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INTRODUCTION

Plants have been used as medicines since ancient times for the treatment of a variety of ailments. In India, herbs are used in traditional medicine and their curative potentials are well documented(Dubey et al, 2004). Therapeutic efficacy of plants for treatment of several disorders has been described in traditional system of medicine. Phytotherapy manuals available have mentioned various medicinal plants for treating infectious diseases like cutaneous infections, gastrointestinal disorders, respiratory disease and urinary tract infections and skin infections. A large number of medicinal plants are identified as antimicrobial agents which are potential in the treatment of microbial infections due to the production of secondary metabolites(Iwu et al, 1999). Plants are rich in a number of secondary metabolites such as alkaloids, flavonoids, phenolic compounds, tannins and phenolic compounds which is found to be active against diseased

condition. A secondary metabolite is no directly involved in normal growth, development and reproduction processes but has other important ecological functions(Ramaswamy Vijayakumar, 2018). Due to increased awareness of hazards caused by pharmaceuticals drugs, there is accelerated investigation and usage of plants as medicine. Moreover the modern drugs are costly and have many side effects. Herbal drugs are considered as alternative medicines to overcome the drawbacks and used strategically for the prevention of disease and promotion of health(Pillai et al, 2012; Compean 2014). Drugs prepared from plants and herbs is in more demand with increased awareness regarding the safety and efficacy of herbal drugs(Jeeyaseelan 2012). Moreover there is increased incidence of antibiotic resistance developed in microorganisms requiring effective treatment. Antibiotics resistance in microbes can cause severe diseases challenging the scientific community to develop new, safe and effective antibiotic compounds from natural sources apart from the existing synthetic antibiotics(Toutati et al, 2018). There is an increased need for the discovery of new and potential drugs to address the new and emerging antibiotic resistant pathogens. Suboptimal usage of antimicrobials for sanitation, nonconformity with infection control practices, continued medication, communicable diseases control practices, relocating the infected patients from one hospital to another, grouping of infected patients for long period of time, antibiotic use in agriculture and domestic stint, increasing national and international tarvel are the reasons for increasing antimicrobial resistance(Cos et al, 2006). The search for plants with antimicrobial activity is important so as to minimize the risk of infectious diseases caused by bacteria, fungi, viruses and parasites which are pathogenic to humans. Plant extracts are major source of many therapeutic agents including antimicrobial agents for the treatment of infectious diseases(Durainpandiyan et al, 2006; Ahmad 2006). Many plants show invitro antimicrobial properties due to the presence of secondary metabolities(Mosihuzzaman et al, 2008). The literature revealed that Trigonella foenum graecum(Sharma et al, 2017), Cinnamomom verum(Gende et al, 2008), Aquilaria malaccensis (Wetwitayaklunm et al,2009), Nigella sativa (Emeka et al, 2015)and Carthamus tinctorious (Sulieman et al, 2018)as herbs possess antibacterial and antifungal activity. Pharmaceutical companies have renewed interest in exploring plants as a major source for lead s in drug development and phytotherapeutics with promising efficacy, quality and safety. The aim of the present study is to screen out a novel polyherbal formulation for antimicrobial activity.

MATERIAL AND METHODS

Plant raw materials:

Five herbs namely Trigonella foenum graecum, Cinnamomom verum(50 g), Aquilaria malaccensis(50 g), Nigella sativa(50 g) and Carthamus tinctorous(50 g) were purchased from local market in Hyderabad and authenticated by Dr. Abdul Samad, A.S clinic, Hyderabad, Telangana, India. Nutrient agar and Sabouraud media for antimicrobial activity were purchased from Standard Chemicals, Hyderabad, Telangana.

S.No.	Botanical name	Common name	Family	Part used	Quantity used
1	Trigonella foenum graecum	Methi	Fabaceae	Seeds	50 gm

2	Cinnamomom verum	Dalchini	Lauraceae	Bark	50 gm
3	Aquilaria malaccensis	Oud	Themelaeaceae	Resin	50 gm
4	Nigella sativa	Kalonji	Ranuanculaceae	Seeds	50 gm
5	Carthamus tinctorious	Karad	Asteraceae	Oil	50 ml

Preparation of Extracts:

All the plant materials (50 g each) were cleaned and powdered separately using a grinder red and stored in an air tight glass container. The powdered polyherbal formulation (PHF) was extracted with petroleum ether and ethanol. Soxlet apparatus was used for hot extraction of PHF and temperature was controlled between 60^{o} – 80^{o}C. The extracts were dried under reduced pressure using rotavapor bath at 40^{o}-45^{0}C. The dried extracts were dissolved in petroleum ether(PHPE) and ethanol (PHEE) in varying concentrations. PHPE1 (25µg/ml), PHPE2(50µg/ml), PHPE3(100µg/ml) and PHEE1 (25µg/ml), PHEE2(50µg/ml), PHEE3(100µg/ml) were prepared using distilled water and stored in capped glass vials.

Evaluation of Phytochemical constituents:

Standard qualitative tests (Kokate et al, 2009)were done to check the presence of phytochemical constituents like alkaloids, carbohydrates, flavonoids, reducing sugars, saponins, sterols and tannins. These secondary metabolites may be responsible for its antimicrobial activity.

Antimicrobial Screening using Agar well diffusion method:

Innoculum and culture media: The subcultures for each microorganism was obtained from the Microbiology Department of Anwarul Uloom college of Pharmacy. The bacterial strains employed were B.subtilis, S.aureus, Shigella dysenteriae, P.aeroginosa and E.coli. The fungal strains used were A.niger and C.albicans.

Methodology for antimicrobial activity:

Antimicrobial assay of PHPE and PHEE was performed by agar well diffusion method (Baur et al, 1996)in Mueller Hinton Agar (MHA) plates. The pure bacterial were subcultured in nutrient broth at 37ºC for 24 hrs. Similarly pure fungal strains were subcultured on Sabouraud's agar(SDA)at a temperature of 28ºC for 3-5 days. Six wells of 6mm diameter were bored with help of sterile cork borer. Each well was filled with 50 µl PHF extract of various concentrations. Sterile DMSO was used as negative control. Ofloxacin (10µg/ml) was used as positive control for antibacterial activity and Ketoconazole (10µg/ml) was used as positive control for antifungal activity. Each well was aseptically transferred with 10µg/ml of different concentrations of PHF. The plates were then incubated at 37 ºC for 24 hrs and 28ºC for 3-5 days for antibacterial and antifungal activity respectively. The plates were observed for the formation of clear zones around the well which indicates the antimicrobial activity of the PHF extracts. The zone of inhibition(ZOI) was observed and measured in mm.

RESULT AND DISCUSSION

The results revealed the presence of alkaloids, flavonoids, saponins, tannins, and glycosides shown in table 1. Phytochemical constituents such as alkaloids, glycosides, tannins, saponins and several other aromatic compounds serve as defence mechanism against several microorganisms, insects and other herbivores (Bonjar et al, 2004). Presence of tannins and

phenolic compounds have been primarily responsible for antimicrobial activity against a number of microorganisms. The bioactive compounds present are known to act by different mechanisms and show antimicrobial action. Tannins are said to bind to proline rich proteins and interfere with protein synthesis. Flavonoids are basically hydroxylated phenolic compounds known to be synthesised by plants in response to microbial infections. Their activity may be due to ability to form complex with extracellular and soluble proteins and to complex with bacterial cell wall(Shibabuddin et al, 2004).

The data showed that the petroleum ether and ethanolic extract of the polyherbal formulation exhibited similar antimicrobial activity at same concentrations against B.subtilis, S.aureus, Shigella dysenteriae, P.aeroginosa, E.coli and A.niger , C.albicans as shown in table 2. The standard drugs Ofloxacin and ketoconazole have better inhibitory effect on bacteria and fungi respectively when compared to the PHF. The highest activity shown by PHEE3 against E.coli and C.albicans. All the ethanolic extracts were shown to possess better antimicrobial activity than the petroleum ether extract of the PHF. The PHEE3 showed substantially significant activity ranging between 21±0.421 mm to 26.83±0.404 mm against all strains of microbes used. And then by PHEE3 ranging between 20.5±0.763 mm to 26.1±.477 mm. In our investigation we found that E.coli and C. albicans are more sensitive to the PHF. This may be due to more susceptibility of cell wall and outer membranes to the PHF extract used. Tannins are known to inhibit many microbial enzymes in raw culture filtrates or in purified forms. The astringent property of the tannins is said to be reported due to its complexation with enzymes or substrates and metal ions(Akiyama et al, 2001). Polyphenols are said to have antimicrobial activity probably due to enzymes inhibition in the oxidised form or by more nonspecific interactions with the proteins. Flavonoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infections were studied extensively and are reported to show antimicrobial activity against many microorganisms(Kumar et al, 2013). The PHF showed significant antimicrobial activity due to the presence of secondary metabolites like flavonoids and tannins that may be contributing to the antimicrobial action. The healing properties of plants are because of the presence of characteristic secondary metabolites namely phenols, flavonoids, alkaloids, etc in them. These metabolites have several bioactivities such as antimicrobial, antioxidant, antihelmintic anticancer etc. Bioactive compounds currently extracted from plants are used as food additives, dyes, insecticides, cosmetics, perfumes and fine chemicals. These compounds belong to a group collectively known as secondary metabolites. Living beings are known to be susceptible to microbial attack followed by multidrug resistance of microorganisms. A number of biological constituents in good yield and some have been shown to possess useful biological actions belonging mainly to phenolics, flavonoids, terpenoids (Sarmistha et al, 2004). Flavonoid has gained recent interest because of their broad biological and pharmacological activities including anti-oxidant, anxiolytic, anticancer, analgesic and antimicrobial. Saponins are common constituents of plants that exhibit a broad spectrum of biological activities and frequently possess hemolytic, cytolytic and bactericidal activities. As the polyherbal formulation is bestowed with almost all the above listed phytoconstituents, the antibacterial activity can be attributed to these constituents.

Table 1: Results for Phytochemical constituents

S.No.	Tests	Petroleum ether	Ethanol
1	Test for alkaloids: 1. Dragendroff's test 2. Wagner's test	 +ve -ve	 +ve -ve
2	Test for carbohydrates: 1. Barfoed's test	 -ve	 -ve
3	Test for flavonoids: 1. Sulphuric acid test 2. Lead acetate test	 +ve +ve	 +ve +ve
4	Test for saponins: 1. Froth formation test	 -ve	 +ve
5	Test for tannins and phenolic compounds: 1. Ferric chloride test 2. Lead acetate test 3. Gelatin test	 +ve +ve -ve	 +ve +ve +ve
6	Test for glycosides 1. Killer killiani's test 2. Salkowski	 +ve -ve	 +ve +ve

Table: 2 Zone of inhibition of different concentration of PHF against bacterial cultures and fungal strain.

Solvent	Zone of Inhibition (mm) MEAN ±SEM						
	Test organism						
	B.subtilis	S.aureus	S.dysenteriae	P.aeroginosa	E.coli	A.niger	C.albicans
Petroleum Ether							
PHPE1	14.8±0.6009	18±0.577	16.83±0.477	15.16±0.500	18±0.638	14±0.557	19.33±0.66
PHPE2	17.1±0.477	19.33±0.421	20±0.577	17.33±0.494	21±0.33	18.66±0.494	22.5±0.5
PHPE3	20.5±0.763	20.66±0.715	23.33±0.614	21±0.577	24±0.730	23±0.557	26.1±0.477
Ethanol							
PHEE1	15.66±0.667	16.5±0.619	16.6±0.619	16.66±0.614	16.66±0.616	17.5±0.341	17.66±0.421
PHEE2	18.66±0.447	17.33±0.494	17.5±0.619	20.16±0.792	20.16±0.79	20.16±0.874	20.83±0.703
PHEE3	21±0.421	23.83±0.477	24±0.516	23.83±0.477	26.83±0.793	25±0.730	26.83±0.404
Standard(Positive Control)							
Ofloxacin	27.66±0.333	33±0.577	27.66±0.494	30.83±0.654	32.16±0.401	-	-
Ketoconazole	-	-	-	-	-	32.16±0.477	35.16±0.66

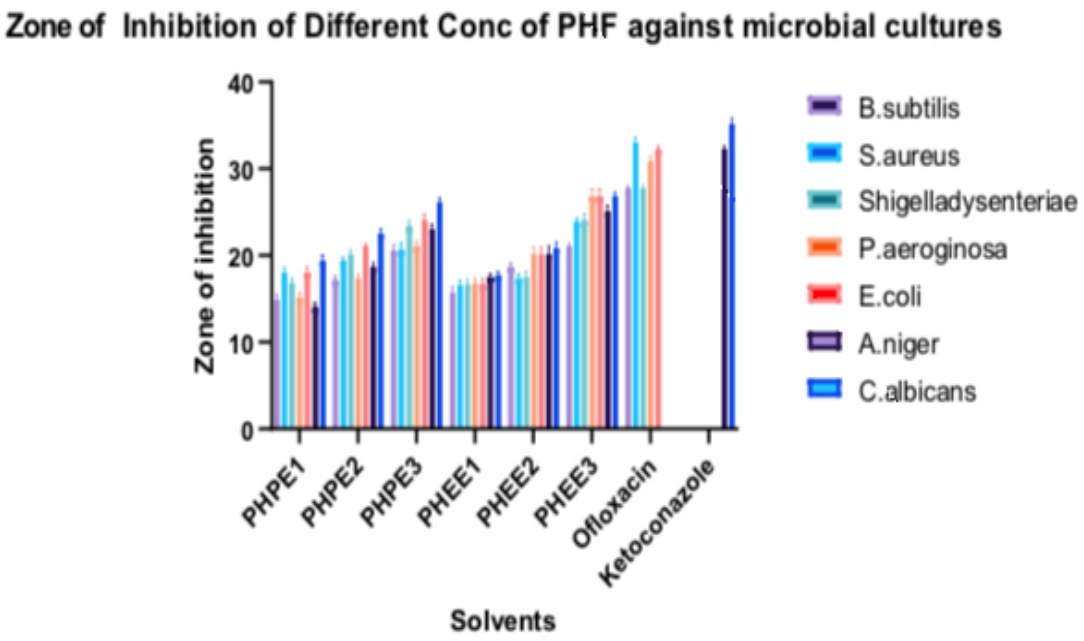

Figure 1: Antimicrobial activity of the PHF

CONCLUSION

The present study confirms the antimicrobial efficacy of the polyherbal formulation developed. As the PHF is a combination of Trigonella foenum graecum, Cinnamomom verum, Aquilaria malaccensis, Azadirachta indica, Nigella sativa and Carthamus tinctorous, it gives a synergistic effect leading to inhibition against pathogenic bacteria and fungi. Moreover, polyherbal formulations are easy to procure, cost effective with minimum side effects. The PHF studied can further be investigated so as to develop more potent bioactive molecules from purified secondary metabolites.

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