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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF CONSTITUENTS OF TRIPHALA

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ABSTRACT

Triphala, a well known ayurvedic formulation, is used against number of ailments since ancient times. It consists of Emblica officinalis, Terminalia chebula and Terminalia bellerica in equal proportions. Triphala as a whole and its three individual constituents show specific antimicrobial activity against certain bacteria and fungi.Triphala is being extensively researched for its various therapeutic effects including its anti-caries, anti-oxidant,anti-colagenase, and anti-microbial activities. Antibacterial activity of aqueous extract of Triphala and its constituents was studies against three human pathogenic Gram negative bacteria namely P.aeruginosa, E.coli and k.pneumoniae and two human pathogenic Gram positive bacteria B.subtilis and S. aureus by cup-plate method. Triphala was found strongly bactericidal against P.aeruginosa with 1.8 cm of inhibitory zone. This was on account of T.chebula, which showed highest inhibitory zone against the same pathogen, followed by E.coli and other two Gram positive bacteria. T.bellerica however, showed maximum inhibitory activity against B.subtilis by showing 2.2 cm of inhibitory zone. It was confirmed that antibacterial activity against Gram negative bacteria was due to T. chebula and E.officinali, while antibacterial activity against Gram positive bacteria was on account of T.bellerica. Antifungal activity of Triphala and its constituents was studied against two pathogenic fungi viz. Aspergillus niger and Candida albicans. Triphala was found almost equally effective against A.niger and C.albicans. Aqueous extract of E.officinalis showed potent antifungal activity against A.niger with an inhibitory of 3.4 cms.

KEYWORDS: Triphala, Emblica officinalis, Terminalia bellerica, Termilalia chebula, Antibacterial activity, Antifungal activity.

INTRODUCTION

Ayurveda, an Indian system of medicine is a holistic science that was discovered several years ago. It is preventive as well as curative.

In developing countries like India, about three fourth populations depend on plant based preparations used in their traditional medicinal system to meet the basic needs for human primary health care (WHO, 2002).[1] Ayurveda is an ancient system of medicine in India, which is based on balancing the three basic elements vat, pitta and kapha. Authentic information on Ayurveda has been compiled by ancient Indian medicine practitioners in forms such as Charak Samhita, Sushruta Samthita etc. Triphala has been described in the ancient Ayurvedic text as Tridoshic rasayana, a therapeutic agent which balances and rejuvenates the tridoshic elements in human body.

The three constituents of Triphala namely Emblica officinalis (Euphorbiaceae), Terminalia chebula (Combretaceae), and Terminalia bellerica (Combretaceae), have various phytochemicals leading to its different medicinal properties including antimicrobial activity. Triphala is being extensively researched for its various therspeutic effects. It is a polyherbal prepration containing tannin, flavanoids, gallic acid, phenols and polyphenols.[2] E.officinalis is rich in tannins and has been reported to have flavanoids, phenols and saponins in both fruit and its extract.[3,4] Fruits of T.bellerica also contain gallic aacid, belleric acid and chebulagic acid.[5,6] The fruits of T.chebula are in tannins e.g. chebulic acid, chebulagic acid and terchebulin.[5] The three individual constituents of Triphala, have been separately to show antimicrobial activity against various fungi as well as Gram positive and Gram negative bacteria.[7]

In this research paper, an attempt has been made to study the antimicrobial activity of Triphla and its various constituents against common human pathogenic bacteria and two human pathogenic fungi. Gram positive bacteria selected for the purpose of study were Bacillus subtilis

and Staphylococcus aureus and Gram negative bacteria were Pseudononas aeruginosa, Escherichia coli and Klebsiella pneumonia. Aspergillus niger and Candida albicans were the two pathogenic fungi selected for the present study.[8]

MATERIALS AND METHODS

Triphala and its constituents were procured from the local market for the phytochemical study. The powder were then processed and used for extract preparations. For aqueous extract, 5 gm of each constituent of Triphala and Triphala as a whole were kept in distilled water and boiled for six hours and then subjected to hot maceration. Later, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 minutes. The supernatant was then collected. After six hours of cooling, the supernatant was concentrated to make the final volume by one fourth of the original volume. The volume was then made up to 25 ml by adding distilled water. It was then autoclaved at 121^0C at 15 lbs pressure and either used immediately or stored at 4^0C[14,15] for further phytochemical and antimicrobial screening. The presence of alkaloids, flavonoids, gallic acid and tannins were analyzed qualitatively. Quantitative estimation of gallic acid was performed by HPLC where as that of tannins was performed by acid titration, in both aqueous and alcoholic extracts of Triphala and its individual constituents.[16]

Gram positive bacteria selected for this study were Bacillus subtilis and Staphylococcus aureus and Gram negative bacteria were Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia. Aspergillus niger and Candida albicans were the two pathogenic fungi selected for the present study. Nutrient media and sourbent dextrose agar were used in culturing and isolation of selected pathogenic bacteria and fungi following the standard methods.[12]

The pathogenic bacterial and fungal strains used in the study were procured from Hamidia hospital pathology laboratory, Bhopal. The pure cultures were maintained by regular periodic sub-culturing and were stored at 4^0C. Antimicrobial assay was done by cup-plate method given by Rose et al. (1939)[13] modified by Kaneria et al.(2009).[12]

RESULTS AND DISCUSSION

All the ingredients of Triphla were evaluated as per WHO guidelines for qualitative phytochemical studies[11] The extracts were analyzed for there phytochemical content. The qualitative phytochemical results are summarized in Table 01.

In the present study phytochemical were found to be present in higher concentration in aqueous extract as compared to their alcoholic extracts. Highest, 7.61% of tannins were found in T.chebula; where as in E.officinalis, T.bellerica and Triphala as a whole, tannins were found to be 4.78%, 5.07% and 3.4% respectively Maximum gallic acid concentration was found in Triphala which was 0.74% where as its minimum concentration was found in T.chebula which was 0.24%.[14] Antimicrobial activity of various constituents of Triphala and Triphala as a whole was observed as a zone of inhibition against individually tested microorganism. Triphala as compared to its constituents was found to be most effective against E.coli by exhibiting 1.7 cm of zone of inhibition. It was followed by T.chebula which showed 1.4 cm of zone inhibition and E.officianalis and T.bellerica each showed 1.2 cm of zone of inhibition.[16,17]

Triphala as a whole exhibited 1.4 cm inhibitory zone against K.pneumoniae demonstrating a synergistic activity of the two constituents against the bacteria as E.officinalis showed 0.9 cm of zone of inhibitition and T.bellerica exhibited 0.7 cm of inhibitory zone. On the other hand, T. chebula did not showed any inhibitory effect against K.pneumoniae. These results are in corroboration with the earlier reports of Javalae and Sabnis (2010) Triphala as a whole showed 1.9 cm zone of inhibition against P.aeruginosa, which was maximum amongst all the five tested bacteria. T. chebula showed 1.2 cm of inhibitory zone against the same pathogen while 0.9 cm of inhibitory zone was exhibited by E.officinalis and T.bellerica each.[18,19]

Comparative account of bactericidal activity of three constituents of Triphala against B.subtilis showed the maximum inhibitory zone of 2.3 cm was exhibited by T.belerica, T.chebula and E.officinalis individually showed 1.5 cm and 0.7 cm zone of inhibition. Triphala as a whole showed 1.7 cm inhibitory. zones as a result of synergistic effect of the three constituents against the same pathogen. S.aureus was found to be most sensitive for T.bellerica by exhibiting 1.7 cm of inhibitory zone followed by T.chebula, Triphala as a whole and E.officinalis by showing 1.2,1.1 and 0.7 cm of inhibitory zones respectively (Table 02 & Fig.02).

Table 01: The qualitative Phytochemical characters of Terminalia chebula,Terminalia belerica,Emblica officinalis.

SR.NO.	PLANT CONSTEUENTS	T. chebula	T. belerica	E.officinalis
01	Alkaloid	-	-	+
02	Glycoside	+	+	+
03	Carbohydrate	+	+	+
04	Gum &Mucilage	+	+	+
05	Tannin	+	+	+
06	Saponin	-	-	+
07	Phytosterol	+	+	-
08	Fat	-	-	-
09	Volatile oil	-	-	-

Table 02: Diameter (cm) of zones of inhibition obtained against five pathogenic bacterial species.

Sr. No	Name of the organism	Triphala	E.officianalis	T.chebula	T.bellerica
01	E.coli	1.8	1.3	1.5	1.3
02	P.aeruginosa	1.9	0.9	1.2	0.9
03	K.pneumoniae	1.5	1.0	0.0	0.8
04	S.aureus	1.1	0.7	1.3	1.7
05	B.subtilis	1.7	0.7	1.5	2.3*

* Maximum inhibitory zone.

Table 03: Diameter (cm) of zones of inhibition against A.niger and C.albicans for Triphala & its constituents.

Sr.No.	Name of the organism	Triphala	E.officianilis	T.chebula	T.bellerica
01	A.niger	2.4	3.5*	1.3	1.7
02	C.albicans	2.3	1.3	1.0	1.6

* Maximum inhibitory zone.

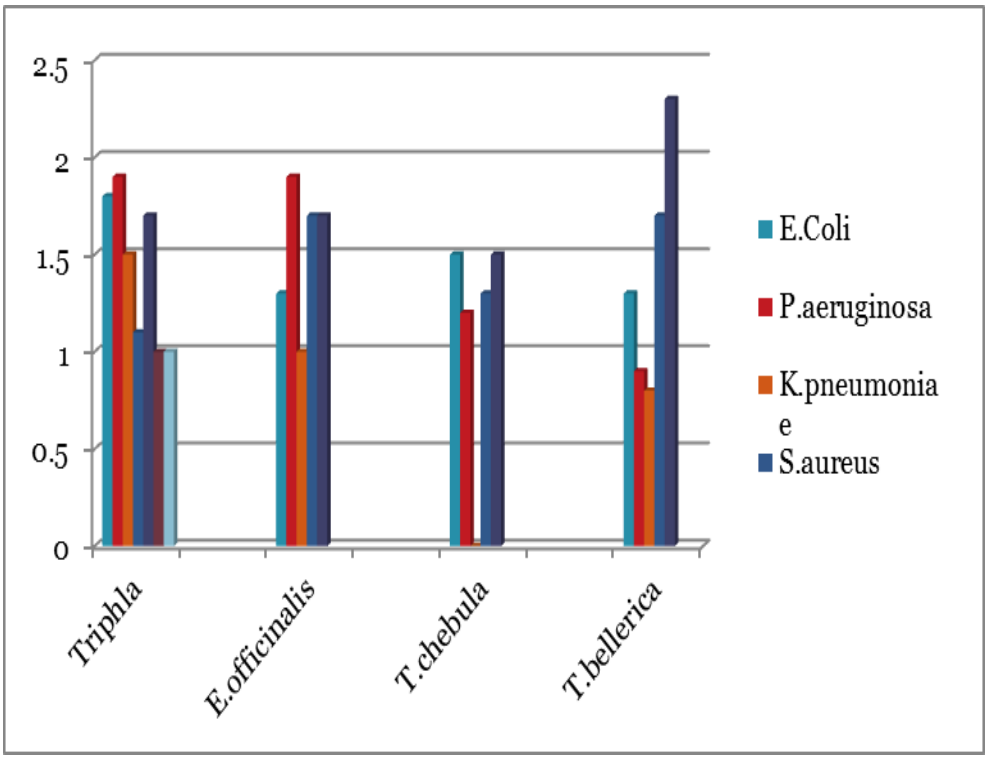

Fig. 02: Comparitive chart of zones of inhibition obtained against all the five pathogenic bacterial species.

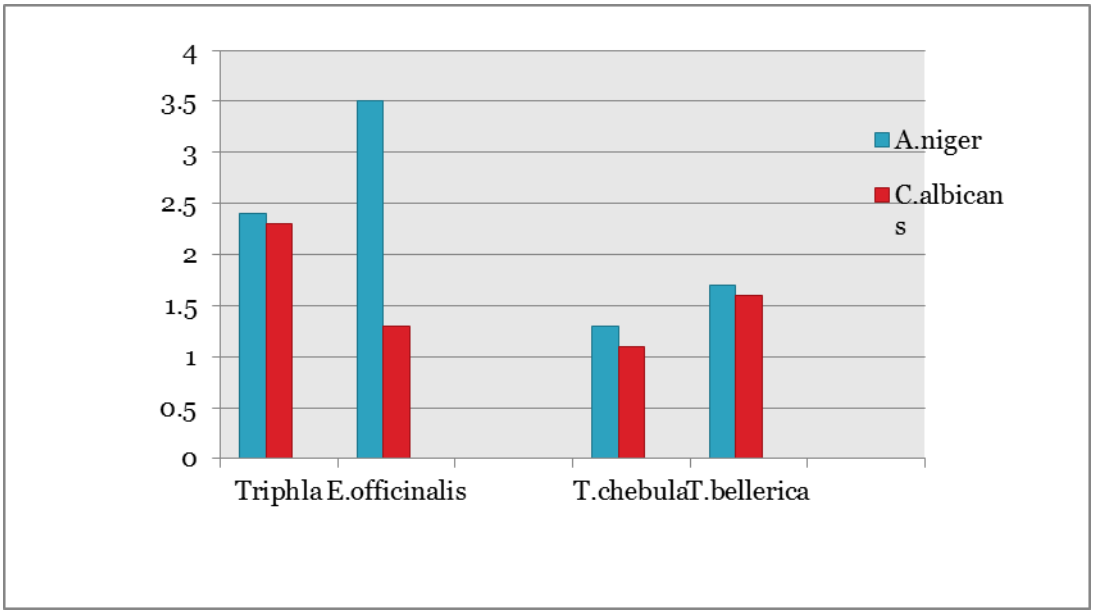

Fig. 03: Comparetive chart of zones of inhibition obtained against A.niger and C.albicans.

Triphala showed 2.4 cm and 2.3 cm of inhibitory zones against A.niger and C.albicans respectively. However, against A.niger, F.officinalis exhibed 3.5 cm of inhibitory zone followed by T.bellerica and T.chebula, which exhibited 1.7 and 1.3 cm of zones of inhibition respectively. On the other hand, T.bellerica showed 1.7 cm of inhibitory zone against C.albicans and E.officinelis showed 1.3 cm of zone of inhibition against it. However, no zone of inhibition was exhibited by T.chebula against the same pathogenic fungi. (Table 3 and Fig 3).

From the aforesaid results it can, therefore, be concluded that there is synergism between the three individual constituents of Triphala against Gram negative bacteria, while its activity against Gram positive bacteria is primarily due to T.bellerica. The antifungal activity of Triphala against C.albicans is due to synergism between E.officinalis and T.bellerica; while that against A.niger is primarily due to E.officinalis. Triphala showed greater antifungal activity as compared to antibacterial activity by exhibiting more than 2 cm inhibition zone.[19,20,21]

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