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STUDY OF THE ANTIMICROBIAL PROPERTIES OF TRIFALA, JAVA PLUM, CINNAMON, HENNA AND CUMIN

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Abstract

The present study was conducted for antimicrobial activity assessment of five selected fresh samples from different conditions. The antimicrobial effects of Tripfala, Java plum, Cinnamon, Henna and Cumin against both gram-positive and gram-negative bacteria were investigated using the agar well and disc diffusion method. Aqueous extracts of selected samples extracts showed larger zone of inhibition or antimicrobial activity than ethanolic extracts. Among the five selected microbes *Klebsiella sp*. showed lowest sensitivities against Tripfala, Java plum whereas these aqueous and ethanolic extracts showed great antimicrobial activities against four other selected microbes. All of the natural products showed the MIC values ranged from 12.5-25.0ug/ml while the MBC values ranged from 25- 100.0ug/ml. No change of antimicrobial activity was found during four months preservation of prepared discs of these natural plant products. This study is an indication that the Tripfala and Java Plum have the potentiality to use as a source for new broad spectrum oral antibiotics.

Key Words: Antimicrobial, microorganisms, *Emblica officinalis*, *Terminalia chebula*, *Terminalia belerica*, *Syzygium cumin*, *Cinnamomum verum*, *Lawsonia inermis* and *Cuminum cyminum*

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1. Main text

In view of increasing resistance to existing antimicrobial agents, some research has been performed worldwide to identify herbal drugs, as they are very important sources for discovering some new agents for treating various ailments related to bacterial infections (Pan et al 2012). With increasing resistance, antibiotics are occasionally associated with adverse side effects to the host, including hypersensitivity, immune-suppression and allergic reactions (Graul et al 2009). These adverse reactions lead to discovering some natural antimicrobial agents which will be effective against pathogenic microorganisms with minimal side effects. One possible strategy is the rational localization of bioactive products from folk medicines, with the hope that systematic screening of these will result in the discovery of novel moiety with potent and useful activities against microbes. With this respect, there is an increasing demand for plant-based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and, in most cases, easily available at affordable prices with no side effects (Winslow and Kroll 1998). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan 1999) and have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Pyrrole pigments, isoprenoid compounds and phenolic plant constituents) (Geissman 1963). Many of the plants could be used as stimulants, hallucinogens or as medicine because of the presence of unique or rich biological-active plant chemicals. Chemicals that make a plant valuable as medicinal plant are: alkaloids (compounds has addictive or pain killing or poisonous effect and sometimes help in important cures), glycosides (use as heart stimulant or drastic purgative or better sexual health), tannins (used for gastro-intestinal problems like diarrhea, dysentery, ulcer and for wounds and skin diseases), volatile/essential oils (enhance appetite and facilitate digestion or use as antiseptic/insecticide and insect repellant properties), fixed oils (present in seeds and fruits could diminish gastric/acidity), gum-resins and mucilage (possess analgesic property that suppress inflammation and protect affected tissues against further injury and cause mild purgative), and vitamins and minerals (fruits and vegetables are the sources of vitamins and minerals and these are used popularly in herbals) (Ghani1998). Triphala ia a mixture of Bibhitaki (*Terminalia bellirica*) with Haritaki (*Terminalia chebula*) and Amla (*Phyllanthus emblica*) which is rejuvenating in nature (the Ayurvedic Formulary of India 2003). Bibhitaki is very useful for hair, throat, and eyes related problems. It is known to boost the immune system of the body and it also helps in maintaining the balance of “Tridosha (humor)” especially Kapha (Water). Bibhitaki, the wonder medicine has several health benefits, e.g.- prevents hair loss and removes dandruff, helpful in constipation, removes acne and ulcers, fights with skin allergies, prevents cough and cold, boosts immunity, prevents diabetes and help in weight loss. Haritaki fruits are highly nutritious for human health as they contain various vitamins, minerals and proteins. They are an excellent source of vitamin C. These fruits are also rich in several minerals including selenium, potassium, manganese, iron and copper. Henna is used as a topical treatment for fungal diseases of the skin, particularly those caused by Tinea (Fariba et al 2010). It is also used to relieve rheumatic pain. A review of experimental animal studies indicates that henna extract enhances wound contraction, reduces epithelialization time, skin cancer chemo preventive activity (Pradhan et. al. 2012, Kapadia et al 2013); it significantly increases the weight of granulation tissue and, thus, its use has been suggested for improving wound healing (Nayak et. al. 2007). This study was based for the scientific evaluation and use of selected natural products or plant materials such as Trifala mixtures of Amla (*Emblica* officinalis), Haritaki (*Terminalia chebula*), and Bibitaki (*Terminalia belerica*), Java plum (*Syzygium cumin*), Cinnamon (*Cinnamomum verum*), Henna (*Lawsonia inermis*), Cumin (*Cuminum cyminum*), which includes study the antimicrobial effect of some natural products (herbal and spice extracts); comparative analysis of antimicrobial activity between aqueous and ethanol extract of test products; and determination of MIC and MBC of these extracts. It was expected that since establishment of project, there might have been some impact on its beneficiaries in respect of their socioeconomic issues.

2. MATERIALS AND METHODS

2.1 Study population (test organisms) Reagent grade nutrient agar (NA) medium was used and both Gram-positive (G+ve) and Gram-negative (G-ve) strains of bacteria were used as the test organism to observe the anti-bacterial activity of the compounds, which were *Staphylococcus aureus* (G+ve); and *Salmonella sp., Shigella sp., Escherichia coli*, *Klebsiella sp.* (all are G-ve).

2.2 Selection, Collection and Size of Samples or plants The selected sample of different parts of plant, herbal, and spice materials were collected from different local areas and market in Dhaka city, Bangladesh. Five natural product materials were used in present study (Table 1). All sample containers were marked immediately and were transport to the laboratory with special polybag within 1h.

Table 1: Name of the different herbal plant and their parts used.

Name of the plants	Parts used
Trifala (Amla+ Haritaki+ Bibitaki)	Fruits
Java plum (*S. cumini*)	Fruits
Cinnamon (*C. verum*)	Bark
Henna (*L. inermis*)	Leaves
Cumin (*C. cyminum*)	Fruits

2.3 Drying, Pulverization and Sample preparation The fresh plant products were first washed with water to remove adhering dirt and then cut into small pieces, dried overnight in Hot air oven at 50°C. After complete drying, the entire portions were pulverized into a coarse powder with the help of a grinder machine and were stored in an airtight container for further use. 10g of the solid sample was weighed aseptically into a sterile jar and 90ml of distilled water was added to it. It was homogenized with sterile blender at 3000rpm for 5-10min. 1ml of homogenate was transferred to a test tube containing 9ml of sterile distilled water to make 10^{-1} dilution and shaken with vortex mixer. A serial dilution up to 10^{-8} was also made in the same procedure.

2.4 Preparation of medicinal plant extracts and Standardization of inoculums In ethanolic process, the powdered 10gm powders were added with 100ml distilled ethanol in a conical flasks. Then the solution was kept in room temperature for 24-72h, after that the homogenate was filtered with a sterile 11cm filter paper, then the extraction was kept in a sterile container and stored at -20°C. In mechanical process, aqueous Trifala, Java plum, Cinnamon, Henna and Cumin extract was prepared according to methods previously reported by (Hoque et al 2008). 1gm of residual ethanol extract powder was mixed with 10ml distilled water and these were kept in room temperature 2-3days. Then homogenate was filtered off with sterile cheese cloth and then 11cm filter paper. Filtrate was directly used for the sensitivity test. A standard stock of the bacteria isolates were prepared by suspending a loop full of each microbial growth in about 5ml of sterile saline. After incubation at 37°C for 12h, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about 5×10^5 cfu/ml (Murray et al 1999).

2.5 Preparation of Test plates and Agar Well preparation Suspensions of the bacterial isolates were made in sterile normal saline and adjusted to the 0.5 McFaeland's standard. Each nutrient agar plate was uniformly seeded by means of sterile cotton swab dipped in the suspension and spread on the agar plate surface, and the plates left on the bench for excess fluid to be absorbed (Murray et al 1999). Agar Wells of 6 mm in diameter, 4mm deep and about 2cm apart were punched in the NA with a sterile cork-borer. Approximately 80µl of the extracts were dropped into each well which filled them respectively to fullness. The setup was allowed to stabilize for 3h before being incubated at 37°C for 24h as described previously (Murray et al 1999 and Aibinu et al 2007). In present study, the aqueous extract concentration of 100µl/well was used.

2.6 Determination of MIC- MBC and zone of inhibition (ZOI) in different concentration The MIC is the lowest concentration of the test sample or drug at which it shows the highest activity against

microorganism. The minimum inhibitory concentration was determined by serial dilution techniques using nutrient broth media. The minimum inhibitory concentration of pure plant was determined against five all of the selected microbes. Dilutions showing no visible growth for the MIC was sub-cultured and incubated at 37°C for 24h. The lowest concentration of the extracts yielding no growth on the NA plate was recorded at the MBC. Serial dilutions 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64 mg/ml (v/v in case of aqueous extract) and (w/v in case of ethanolic extract) were prepared from stock solutions. Then their antimicrobial activities were determined by agar well diffusion procedure.

3 RESULT

3.1 Antimicrobial activity It has been observed from that the aqueous extract of Triphala, Java plum, Henna, Cumin and Cinnamon had good antimicrobial activity against all the selective microbes except *Klebsiella sp.* But the aqueous extract of Cinnamon had not any antimicrobial activity against *S. aureus*. Whereas Cumin showed less sensitivity and Triphala, Java plum, Henna and Cinnamon showed higher antimicrobial activity. It was found that *Klebsiella sp.* was resistance to all the ethanolic extracts except in cinnamon (Table 2). Different concentrations of all the samples were applied in different selected microbes (cfu 1.4×10^7) and the ZOI assayed (Table 3). It was observed that more or less 1:8 concentration showed better result and in the 1:16, the ZOI started to decreased and 1:64 it showed almost negative result. In case of ethanolic extraction, Triphala, Java plum, Henna, Cumin and Cinnamon were tested for their antimicrobial activity against all of the selected microbes. The ethanolic extract was neutralized for the determination of the effect of pH (ethanol) on antimicrobial activity.

Table 2 Sensitivity and resistance pattern of the organism against test products.

Name of organisms	Name of products									
	Triphala		Java plum		Henna		Cumin		Cinnamon	
	S	R	S	R	S	R	S	R	S	R
Salmonella sp.	+	_	+	_	+	_	+	_	+	_
Shigella sp.	+	_	+	_	_	+	+	_	+	_
S. aureus	+	_	+	_	+	_	+	_	_	+
E. coli	+	_	+	_	+	_	_	+	+	_
Klebsiella sp.	+	_	+	_	+	_	+	_	_	+

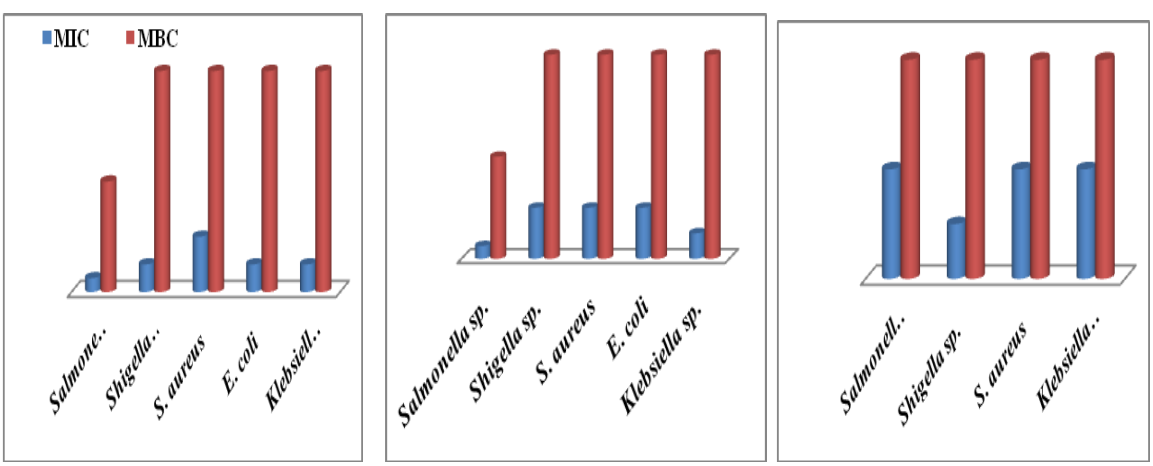
*S= Sensitive; R= Resistant

Table 3 Determination of antimicrobial activity of Triphala, Java plum, Henna, Cumin and Cinnamon in different concentration against different microbes.

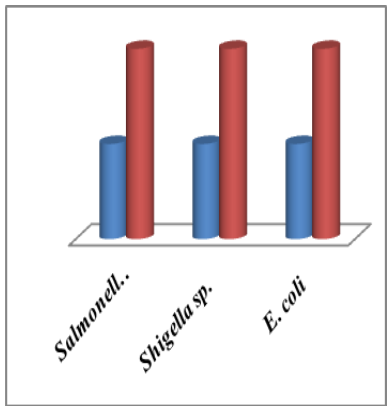
Different Concentration	Name of the Product	Name of the Organisms (ZOI)				
		Salmonella sp.	*Shigella sp.*	*S. aureus*	*E. coli*	*Klebsiella sp.*
Neat	Triphala	35	23	29	25	30
	Java plum	30	21	25	20	29
	Henna	21	-	19	18	19
	Cumin	13	-	14	12	11
	Cinnamon	11	13	-	12	-
1:2	Triphala	26	19	21	19	22
	Java plum	27	17	19	16	23
	Henna	16	-	13	12	14
	Cumin	9	-	10	9	8
	Cinnamon	7	9	-	8	-
1:4	Triphala	21	14	16	13	18
	Java plum	21	11	12	12	17
	Henna	10	-	8	8	10
	Cumin	4	-	7	5	5
	Cinnamon	5	4	-	5	-
1:8	Triphala	16	9	11	9	14
	Java plum	17	7	8	8	13
	Henna	5	-	4	5	4
	Cumin	0	-	4	0	0

	Cinnamon	0	0	-	0	-
	Triphala	12	4	7	4	10
1:16	Java plum	13	4	4	3	9
	Henna	0	-	0	0	0
	Cumin	0	-	0	0	0
	Cinnamon	0	0	-	0	-
1:32	Triphala	8	0	4	0	7
	Java plum	9	0	0	0	4
1:64	Triphala	0	0	0	0	0
	Java plum	0	0	0	0	0

3.2 MIC and MBC It was observed that the MIC of Triphala against *Salmonella* sp., and *E. coli* were 12.5µg/ml; whereas MIC against *Shigella sp*. and *S. aureus* were 50µg/ml and 25µg/ml, respectively. The MBC of Triphala against *Salmonella* sp., and *E. coli* were 50µg/ml and 100µg/ml against *Shigella* sp., *S. aureus* were observed all other organisms. It was observed that the MIC of Triphala against *Salmonella sp*. was 6.25µg/ml whereas MIC against *Shigella sp., E. coli, Klebsiella sp.* were 12.5µg/ml; *S. aureus* 25.0µg/ml. The MBC of Triphala against *Shigella sp., S. aureus, E. coli,* and *Klebsiella sp.* were 100µg/ml and 50µg/ml MBC was observed against all other organisms. In case of Java plum, MIC against *Shigella sp., S. aureus,* and *E. coli,* were 25 µg/ml, MIC 6.25 µg/ml to *Salmonella* sp.; and MIC of 12.5µg/ml were observed in case of other microbes (Fig. 1). On the other hand, MBC of 100µg/ml were observed against all other microbes except *Salmonella* sp. where it was 50µg/ml MBC. The MIC of Henna against *S. aureus, E. coli*, were 50µg/ml whereas *Salmonella sp., Klebsiella sp.* were 12.5 and 25µg/ml respectively. MBC was found 100µg/ml against *S. aureus, E. coli* and *Klebsiella sp.* were found 100µg/ml and 50µg/ml MBC was against *Salmonella sp.* In case of Cumin, 50µg/ml of MIC was against *Salmonella sp., S. aureus,* and *Klebsiella sp.* observed whereas against *Shigella sp.* MIC was 25µg/ml. MBC was found 100µg/ml against all microbes. 50µg/ml of MIC was against *Salmonella sp., Shigella sp.,* and *E. coli* observed whereas the MBC was found 100µg/ml against all selected microbes against Cinnamon (Fig. 2).

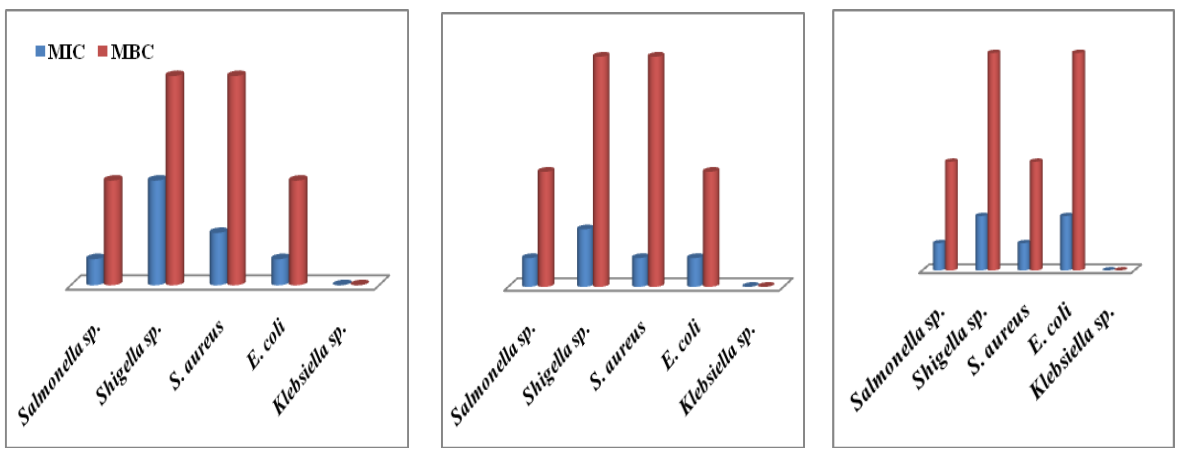


(A) **(B)** **(C)**

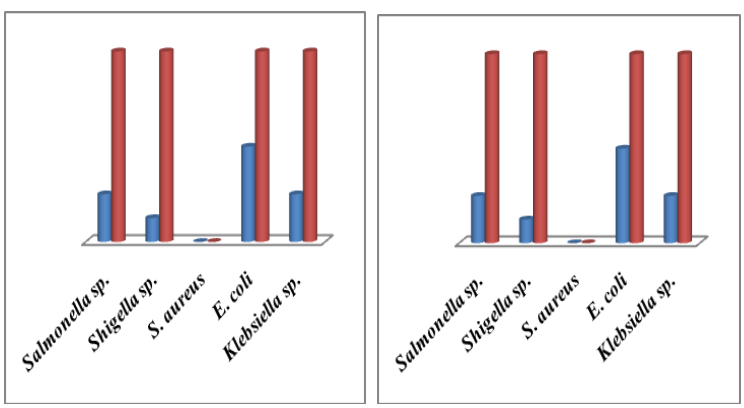


(D)

Figure 1 Determination of MIC and MBC (natural extract) of (A) Triphala (B) Java plum (C) Cumin (D) Cinnamon against different microbes.



(A) **(B)** **(C)**

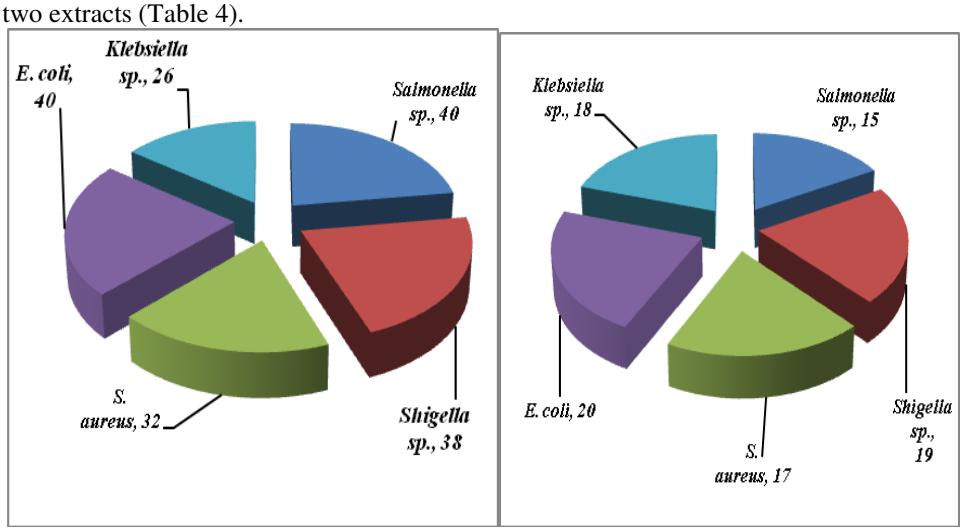


(D) **(E)**

Figure 2 Determination of MIC and MBC (ethanolic extract) of (a) Triphala (b) Java plum (c) Henna. (d) Cumin and (e) Cinnamon against different microbes.

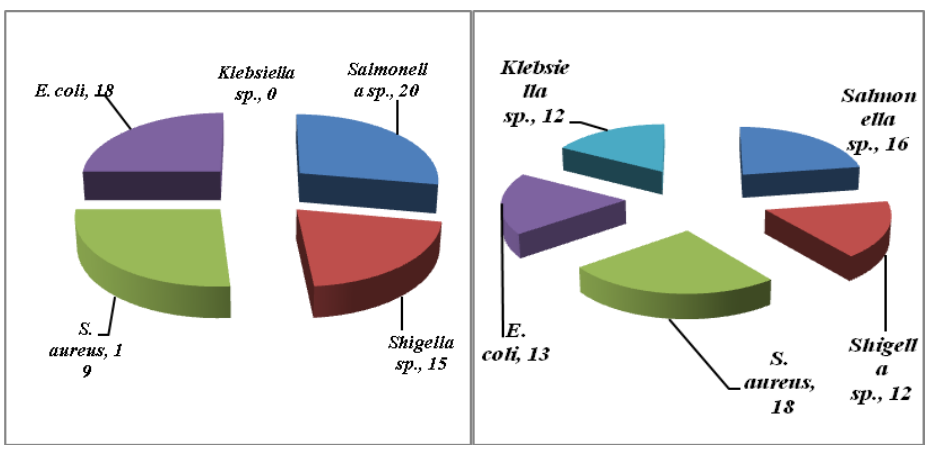
3.3 Combined effect In natural, Java plum+Cumin showed satisfactory results against all the selected microbes except *Klebsiella* sp. (Fig. 3). In case of ethanolic extracts, Java plum+Cumin and Triphala+Cinnamon showed satisfactory results against all the selected microbes (Fig. 4). Only, *Klebsiella* sp. which did not show better result in case of Java plum+Cumin. A Comparative analysis of ZOI of neutral and crude ethanol extraction against test organisms was also done to know the efficacy of

these two extracts (Table 4).



(a) **(b)**

Figure 3 Combined antimicrobial effect (natural extract) of (a) Java Plum +Cumin and (b) Triphala +Cinnamon against different microbes



(a) **(b)**

Figure 4 Combined antimicrobial effect (ethanol extract) of (a) Java plum+Cumin and (b) Triphala+Cinnamon against different microbes

Table 4 Comparative study of ZOI of neutral and crude ethanol extraction against test organisms

Name of organisms	ZOI (mm)									
	Triphala		Java plum		Henna		Cumin		Cinnamon	
	Neutral	Crude	Neutral	Crude	Neutral	Crude	Neutral	Crude	Neutral	Crude
Salmonella sp.	15	26	20	27	11	21	14	24	11	20
Shigella sp.	10	17	11	17	10	18	9	18	13	20
S. aureus	12	22	12	22	9	13	11	22	0	0
E. coli	9	16	10	18	11	17	10	17	9	15
Klebsiella sp.	0	0	0	0	0	0	0	0	10	17

4 DISCUSSION

The screening and isolation of bioactive compounds from plants is increasing, as these compounds are much safer and less toxic than the chemically synthesized drugs. The increasing trends in emergence of resistance among infectious agents, treatment failures and recurrences of infection had led to the search of biologically active compounds or products from plants. The antibacterial activity of Triphala has already been documented. The extract of triphala shows antimicrobial activity against some of the oral bacterial species tested (Srikumar et al 2007). Hence the study was done to evaluate the antibacterial effects of Triphala, Java plum, Henna, Cumin and Cinnamon against some of the bacterial pathogens. The aqueous and ethanolic extract of seeds of Java plum was tested for their activity against five different species. The extract showed great activity against *Salmonella* sp*., Shigella* sp*., S. areus* and *E. coli* but *Klebsiella sp*. whereas was inhibited. The aqueous extract of the entire sample showed more inhibition than the ethanolic extracts against all the species of bacteria that were inhibited. The aqueous extract of samples showed more inhibition than the ethanolic extracts against all the species of bacteria that were inhibited. Antimicrobial effect of aqueous extract of samples were higher than the against *Salmonella* sp. The antimicrobial effect of Triphala may be due to the presence of secondary metabolites, one of the compounds, Gallic acid, Chebulagic acid and Chebulinic acid was found to possess antimicrobial effect on oral bacteria's. Hence further analysis of the compounds and their testing against microorganisms would help to develop a new antimicrobial agent that may be of great importance. The results for the antibacterial screening have shown that all the extracts in this present study have antibacterial activity. The results of the inhibition of bacterial growth have shown that the extracts are active at high concentration and inactive at very low concentrations. Thus the study may suggest that the inhibition of bacterial growth activity of the extracts is dose dependent.

It was observed that the aqueous extracts of triphala showed remarkable activity against all the tested organisms with the highest activity found on *Salmonella* sp. (35mm zone diameter) at 100 µg/well concentration. ZOI showed against triphala was satisfactory antimicrobial activity. Srikumar et al (2007) worked on the same (triphala) and found no ZOI against triphala. This is may be due to the species difference or the triphala difference in different biologic condition. Aqueous extract of Java plum was also showed a good activity against all the tested organisms. (Aparna et al 2015) showed satisfactory activity of Java plum against *S. aureus*, *Staphylococcus saprophyticus*, *E. coli*, *Pseudomonas aeurioginosa* and *Proteus vulgaris*. But in present study it was more effective result was found against all the selected microbes. In case of henna, cumin, it showed antimicrobial activity against all the selected microbes but the ZOI were not satisfactory like Triphala and Java plum. On the other hand, the ethanol extract of Triphala is also active against the test organisms with highest activity on *Salmonella sp.* (26mm) at 100 µg/disc, but (Srikumar et al 2007) observed that ethanol extract of triphala showed highest (16mm) activity on *S. aureus, V. cholera,* and at *E. coli* at 0.1µg/ml. In case of Java plum satisfactory results were found against all the microbes which demonstrate almost same results as the observation by (Cowan 1999) where pronounced activity of Java plum were demonstrated against ethanolic extract where The MBC of the *S. cumini* extract was 62.5µg/ml for the bacteria *S. aureus.* This may be due to the species difference or difference in different biologic condition. Ibukun et al (2007) reported sensitivity pattern of different microbes against ethanol extraction of triphala were *E. coli* 33mm, *Klebsiella sp.* 30mm zone diameter, whereas in present study, no ZOI was observed against *Klebsiella sp*. but other tested organisms. This difference of observation between and present study may be due to heating of triphala in experiment or species differences. *Salmonella sp.* and *S. aureus* showed better effective results in of cumin against different microbes than the present study which might be due to species difference or concentration variation. In case of combination of Java plum + Cumin showed ZOI was 14mm against *Shigella sp*. and *Klebsiella sp.,* whereas in present study, range of ZOI against the selected microbes was observed from 14-22mm (Ibukun et al 2007).

Solvent extracts of Triphala and Java plum showed a remarkable antibacterial activity on almost all test organisms. In this study, triphala (aqueous extract) showed MIC from 6.25 to 25µg/ml. on the other hand,

Gram-positive bacteria were more susceptible to the inhibitory effects of the plant extract. Best activity sensitivity showed against *Salmonella sp.* in aqueous and ethanol extract. *S. aureus* showed best sensitivity in ethanol extract and *Klebsiella sp.* showed no sensitivity in all extract. The antibacterial activity attribute to its chemical constituent as reported by many researches such as Hydroxychavicol. Phenolic compounds such as cavicol, cavibetol, carvacrol, eugenol, and allipyrocatecho (Fabricant and Farnsworth 2001, Beuchat and Golden 1989). In this experiment G+ve bacteria more sensitive than G-ve bacteria and show this result for cell membrane permeability. G+ve bacteria have single layer and lack the natural sieves effect against large molecule. On the other hand G-ve bacteria have multi –layered and complex cell wall structure (Hawkey 1998). Organic extracts were more potent than aqueous extract for two reasons: because of the nature of biological active components enhanced in the presence of solvent such as stronger extraction capacity of solvent produced greater number of active constituents responsible for the antibacterial activity; and betel leaf extract is rich in less polarity phenol compound (ethanol and metabolic) which were more efficient in extracting the active compounds from this species due to its no polar components (Brković et al 2006, Cowan 1999*).* Low/negligible antimicrobial action of aqueous extracts ascribed to low level of the anionic components (Sukanya et al 2009*).* No inhibition was observed with negative controls, which proves that solvents could not act as antibacterial agents. MIC value for G-ve > G+ve bacteria; higher dose of gram-posiative bacteria will be required to cause infection. *Staphylococcus aureous* were found to be most sensitive (MIC: 0.78 mg/l*);* others >more resistant (>1.25mg/l). MIC<MBC value; plant extracts were bacteriostastic at lower concentration and bactericidal at higher concentration. Low MIC exhibited by this plant extract showed that they can be used as an alternative to orthodox antibiotics. No inhibition was observed with controls, which proves that solvents could not act as antibacterial agents. All the test organisms were multi drug resistant. There are all organisms are multidrug resistant means drug (antibiotic) not work against bacteria, but this two natural product work against bacteria. Combined effect of Triphala and Cinnamon was also good but not as good as combination of Java Plum and Cumin showed. The highest ZOI was 20 mm given by *E. coli* and the lowest ZOI was 15mm given *Salmonella sp*.

Extracts of Triphala, Java plum, Henna, Cumin and Cinnamon showed effective antimicrobial agent against all the tested pathogens in controlling their growth in vitro in culture condition. They all had a bacteristatic and bactericidal activity against broad spectrum of microorganisms (Gram-positive and Gram-negative bacteria) when tested in vitro using crude preparation. All of the natural plant products (aqueous and ethanolic); Triphala and Java plum (aqueous extract)were found best antimicrobial activity and both of them could be able to inhibit the growth of all the sensitive microbes. In case of ethanolic extracts of these natural plant products, it was interestingly found that they had antimicrobial activity in all the cases. The microbes that were selected in this present study can cause different types of diseases. Although in some cases, there is need for detailed scientific study of traditional medical practices to ensure that valuable therapeutic knowledge of some natural plants is preserved and also to provide scientific evidence for their efficacies. Also another study will be needed to establish the exact component or pharmacological standardization and clinical evaluation in Triphala.

5. CONCLUSION

As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs from Java plum should be emphasized for the control of various diseases. In fact, time has come to make good use of centuries-old knowledge on Triphala and java plum through modern approaches of drug development. Locally available and can be easily cultivated a good choice for the development of new strategies for therapy of bacterial diseases. Quite a significant amount of research has already been carried out during the past few decades in exploring the chemistry of Triphala, java plum, henna, cumin and cinnamon. This present study may generate enough encouragement among the scientists in exploring more information about these medicinal plant and plant products.

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