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Evaluation of Antibacterial Property of Medicinal Liquid Soap having *Justiciaadhatoda*L.(Adhatoda)Leaf Extracts

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INTRODUCTION

Soaps are the products of basics aponificationreactions (Chukwulozie, 2014). Theyare in useamong society from its discovery by the ancientBabylonians as acleansing material(Warra,2010).Currently,therearealarge numberofcommercially available medicinal soapswhich come mainly as solid bar or liquid soaps. The difference between solid and liquid soaps isthatinsolidsoapNaOH is used as base,whereas in liquid soapsKOH is used as base in there preparation (Chukwulozie,2014).

Different types of naturalingredients are used to enrich the soaps medicinalpropertieslikeantiviral, antibacterial,antifungal,antioxidant,anti-inflammatoryetc.tothefinalproduct(Kole,2005).Besides these, manynaturalingredientsare also used to improvethecolor,textureandodorofthesoapproducts.During preparation of medicinal soap products, it is most importantanddesirable alsotousenaturalingradients instead of harmfulsyntheticchemicals,toobtainthedesired bioactivities. Both BHT (2,6-ditert-butyl-4-methylphenol)and Triclosanareexamplesofsyntheticcompoundswhichhavebeenmostly used in such consumer soap products to giveantioxidant and antibacterial properties respectively.However,these synthetic substances could be replaced bynaturalingredientsobtainedfromcommonmedicinalplants. Turmeric, sandalwood, neem, jasmine and lemonessencearesome ofthemostcommoningredients usedindifferent skincareproductsincludingmedicinal soaps (Kole, 2005). In present study,effortsweremadetopreparemedicinalsoapproductsby usingbiologically activeleafextractsof*Justiciaadhatoda* L.

*Justiciaadhatoda*L.is an evergreen shrub, whichusuallygrowswellinlowmoistureareasanddry soils to a height of 2-3 meters. Its leaves aresimple, opposite, large and lance shaped. TheplantisnativetoIndian subcontinent andare distributed incountrieslikeSriLanka,India,NepalandPakistan(Sampath,2010).*Justiciaadhatoda*L.contains a

varietyofalkaloids, saponins, flavonoids, tannins and phenolics. Quinazolinealkaloid ,vasicine is an impotant constituent of it.Lycopenes,ascorbicacidetc are the other important compounds present here.(Wankhede,2015).

Adhatoda is a well-known drug in folk and Ayurvedicmedicines. It has been used for the treatment of the respiratory tract diseases and disorders like asthma, bronchitis and cough because of its bronchodilatory action (Sampath, 2010). During the last 25 years, several researches on abortifacient and oxytocic effects of quinazoline alkaloid vasicine and other alkaloids derived from the plant have also been reported. Use of leaf juice tocure diarrhea and dysentery is common is rural areas. *Justiciaadhatoda* L. isalso known to possess antibacterial, antioxidant,anti-diabetic,anti-hemorrhagic,anthelminticandanti-rheumaticproperties(Sampath,2010).Inthepreparationofmedicinalsoaps, the applicability of *Justiciaadhatoda*hasbeenreported by Wijetunge&Perera (2015).Adhatodaiswildly distributed,cultivatedandreadilyavailableplant, therefore itcanbesuitable for an industrial level application.Duringthisstudy,optimizedconditions for obtaining bioactive leaf extracts from *Justiciaadhatoda*wereobserved andtheseextractsobtained,weremixed intosoapproductstoimprovetheirmedicinalvalues.

MATERIALSANDMETHODS

The plant parts were collected from different parts of mid- Gangetic plain areas and all are crossed checked by Herbarium of National Botanical Research Institute Lucknow.Mature leaves of *Justiciaadhatoda*leaves obtained fromGorakhpur and adjoining areas were dried and crushed into powder form. The extractions were peparedbymaceration,soxhletextractionandsonication.During this processmethanol,ethylacetateandhexane were usedasthesolvents.

Maceration

A weight of 5 gm. of powdered leaves wasadded with 100 mL volume of the solvent in aconicalflask.Thenthis liquidwaskeptinanorbital incubator for 24 hours at a temperature of 37 ˚C. Thereafter, itwasfilteredby usingaWhatmaan No.1filterpaperfollowedbydryingwithanhydrousNa_2SO_4.Now thefiltrate was collected and then it was concentratedby using rotary evapotaor. After this solventevaporationwas done bywaterbathmaintainedat about50˚C.

Soxhletextraction

10 gm of extremely grounded leaves wereadded to the nozzle of the soxhlet apparatus. Now avolumeof200mLofthesolventandfewboiling stones were added into the rounded bottomflaskandattachedtotheextractorapparatus.Extractionwasdonefor a period of3hours.Then theresulting solutionwas filtered by using Whatmaan No. 1 filter paper followed by drying withanhydrousNa_2SO_4.Now thisfiltratewasconcentrated by usingarotaryevaporator andfollowedbyevaporationofsolventusingawaterbathmaintainedata temperature of 50˚C.

Sonication

5 gm. of extremely crushed leaves wereadded into 100 mL volume of the solvent in aconicalflask.Theextractionwasperformed inasonicator for a period of 2 hours at normal room temperature. Theextracted solutionwas filtered by using a Whatmaan No. 1 filter paper followed by drying withanhydrousNa_2SO_4.Now the filtratewasconcentrated by usingrotaryevaporatorfollowedbyevaporationofsolventusingawaterbathmaintainedata temperature of 50˚C.

Investigationof Antibacterial activity ofAdhatodaleaf extracts

With known concentrations of the prepared plant extracts and the controls, thesterilizedfilterpapersweresoaked into plates of 6 mm diameter.As positive control antibiotic gentamycin (1 mg/mL)was usedand methanol was used asthenegativecontrol.Theplatediffusionassays were carried out against*Salmonellatyphimurium*,*Escherichiacoli* ,*Bacillus cereus* and S*taphylococcus aureus*.

Few drops ofsterilized0.9%NaClsolutionwasinoculatedwitheachbacterial strain separately. These were matched with the turbidity ofthe0.5McFarlandsolutionandtheobtained inoculums were used to prepare bacterial spreads on plates. After about 5 minutes, the impregnatedplates were placed on the spread plates. Theseplates were incubated overnight at about 37 ºC. After the incubation period,thediameters ofthe eachinhibitionzonesweremeasured.Alltheseexperimentswereperformedintriplicate(Driscoll,2012).

Preparationofliquidmedicinalsoaps by usingbioactiveadhatoda extracts

By dissolving 1 gm ofcommercialsoftsoapin10mLofdistilledwater, liquid soap was prepared andaddingacalculated

amountoftherelevantAdhatodaextract.Adhatodasoapwaspreparedtocontaina50mg/mLconcentrationof the methanolicsoxhletextract.whereastheantioxidant soap was prepared having a 10mg/mLconcentrationoftheethylacetatesoxhletextract(Wongthongdee&Inprakhon,2013).

Determinationoftheantibacterial activity ofthepreparedmedicinalsoaps

Sterile filter paper plates were soaked withthe liquid medicinal soap. Liquid soap having noplantextractswereusedasthenegativecontrol. The plate diffusion assays were performedthrice to check the antibacterialactivity of the prepared liquid antibacterial soapcomparedtothatofthecontrolsoap.

Effectiveness test of liquidmedicinal soap

Thumb impressions of handsexposed tothe environment were made on a MHA (MullerHinton Agar) plate with appropriate distance. After this,onethumbwaswashedwiththeprepared liquidmedicinal soap and the other thumb with control soap.Then thethumbimpressionsoftheproperly washedhands were placed on the same MHA plate at asuitablelocationswithoutanyoverlapping. Nowtheseplates were incubated at 37 ˚C for about 24 hours andthe pattern of microbial growth was studied.(Kaur,2014).

Tests for different bioactive compounds in plant extracts

i. Testforalkaloids

A fewdropsoftheHager'sreagent(saturatedsolution of picric acid) was added into a small amount of plant extract. Yellow colored precipitatewas obtained which confirms the presence ofalkaloids(De,2010).

ii. Testfortanninsandphenoliccompounds

A few drops of 5 % $FeCl_3$ was added into a little amount of prepared plant extract. Blue greencolor was appeared which confirms the presence of tanninsandphenolic compounds(De, 2010).

iii. Testforreducingsugars

Fehling's A and Fehling's Breagents were mixedwith each other in equal volume to preparethe Fehling's reagent. A small amount of theFehling's reagent was treated with few drops of plant extract and boiled. Abrick red precipitate is obtained which indicated the presence ofreducingsugars(De,2010).

iv. Testforsteroidsandterpenoids

A little ammountoftheplantextractwastreatedwithasmall volume of chloroform and few drops ofconc.Sulfuric acidwasadded.This mixturewasshakenwellandallowed tostand forfew moments.Redor reddishbrowncolor is observed atlowerorganiclayer indicatedthepresenceofsteroidsandterpenoids(De,2010).

v. Testforsaponins

A small amount of the plant extract was added to few drops of distilled water and well shaken. Astablefroth was observed whichindicatedthepresenceofsaponins(De, 2010).

RESULTSANDDISCUSSION

The present work was mainly focused on extractingbioactive fractions from*Justiciaadhatoda*leavesto prepare medicinal soap products. During theextractionofnaturalbioactive compoundsfromadhatoda,different percentage yields were observedfrom the crude plant extracts obtained by differentextraction processes and solvent combinations.(Table1)

The polarity of solvent decreases from methanol toethyl acetate to hexane. While considering thepercentage crude yields of these three solvents, ingeneral, the polar methanolic extracts performed relativelyhighcrudeyieldspercentagesascomparedtotheothersolvents. The percentage crude yield alone isnota bestindicatorforthepresenceofbioactivecompounds, sotheplantextractswerefurtherexaminedfortheirantibacterialactivities.

Table 1. Percentage yields of different fractions of the crudeAdhatodaleafextracts

Solvent	Percentageyield(%)		
	Maceration	Soxhlet extraction	Sonication
Ethyl acetate	02	07	03
Hexane	01	02	05
Methanol	14	06	14

InvestigationoftheAntibacterialactivity of Adhatodaextracts

All the preparedadhatoda extracts were screened for their antibacterial activity andtheresultsarelistedinTable2.

According to the results obtained, onlythe methanolic extract of adhatoda obtained bysoxhletextractionshowedinteresting antibacterialactivityagainstallthefourbacterialstrains tested at25mg/mLconcentration. The other adhatoda leaf extract didnot show any significant antibacterial action against any of the tested pathogens at 25 mg/mLconcentration.

From above observation it is established thatsoxhletextraction of leaves in methanol is the bestextractionconditiontoextract antibacterialfraction from adhatoda. This fact indicates thatthe antibacterial compounds are polar in nature.Therefore, themethanolicsoxhletextractofadhatoda leaveswasselectedforthepreparationofantibacterialadhatodasoap.

Table 2. Antibacterial assay results for the 25mg/mLadhatodaextracts.

Extraction method	Solvent	Diameterofzonesofinhibition(cm) ± SEM			
		B.cereus	*S.typhimurium*	*S.aureus*	*E.coli*
Maceration	Methanol	NI	NI	NI	NI
	Ethyl acetate	NI	NI	NI	NI
	Hexane	NI	NI	NI	NI
Soxhlet	Methanol	1.8 ± 0.0	1.8 ±0.1	1.5 ± 0.1	0.9 ± 0.0
	Ethyl acetate	NI	NI	NI	NI
	Hexane	NI	NI	NI	NI

Phytochemicalstudies

Themethanolandethylacetateextractsofadhatodaobtainedbysoxhletextractionwereexamined for the phytochemicals presentin each extract. The methanol soxhlet extract ofadhatoda showed the presence many phytochemicals whereas the ethyl acetate soxhletextract showed only the presence of phenols andtannins.(Table3)

Table 3. Phytochemical analysis of methanoland ethyl acetate extracts of adhatodaobtainedbysoxhletextraction.

Solvent	Alkaloiods	Phenols andtannins	Terpenoids	Steroids andterpenoids	Saponins
Methanol	+	+	+	+	-
Ethyl acetate	-	+	-	-	-

Adhatoda is well known to have alkaloids such asvasicine and vasicinone. These are responsible foritsantibacterialactivity(Pa&Mathew,2012).Therefore,thesignificantantibacterialcharcter has beenobservedinthemethanolsoxhletextractof adhatoda. (Table2)It couldbeattributedtothepresence of alkaloids in the methanolic extract asindicatedabove.Accordingto the phytochemicalresults,boththeextracts containedphenolsandtannins.

Thephytochemicalanalysisindicatedthepresenceofimportantsecondarymetabolitesthatcouldberesponsiblefortheobservedantibiotic activities.Theseextractsshowedtheirability to serve as biologically active extracts thatcouldbeincorporatedinthepreparationofmedicinalsoapproducts.

Preparation and evaluationofadhatodasoapproducts

Twomedicinalsoapswerepreparedwithbothbioactiveextracts(methanolsoxhletextractandethylacetatesoxhletextract) .Acontrolsoapwaspreparedwithouttheadditionofextracts.Theantibacterialactivityofthepreparedantibacterialadhatodasoapwasinvestigatedusing the disk diffusion assay and the resultsobtainedareshowninTable4.

Table 4. Antibacterial activity of the soapproducts.

.Sample	Averagediameterofinhibitionzones(cm)			
	B. cereus	*S. aureus*	*S. typhimurium*	*E. coli*
Antibacterial soap	1.0 ± 0.1	1.0 ± 0.0	NI	0.7 ±0.0
controlsoap	1.0	0.7	NI	0.7 ±0.0

	±0.1	±0.0		
Gentamycin	2.0 ±0.1	2.0 ±0.0	2.0 ±0.0	1.4 ±0.1

Eventhougha25mg/mLconcentrationofthe methanolicsoxhletextractofadhatodashowed an antibacterial activity as the pure extract. The concentration was doubled of the extract used to preparethemedicinalliquidsoaptomake certainitsactivity in the final product. Theaddition of the methanoilc extract into the soapproduct was active only against *S. aureus*withaninhibitionzoneof1.0±0.0cmatthe50mg/mLconcentrationoftheextract(Table3).Theantibacterial components in the extract might nothave been stable in the liquid soap medium, so theconcentration used for the preparation of the liquid soapproduct might not have been as enough to inhibit thegrowthoftheotherthreebacterialspecies.However,thegrowthofthesespecieswasretartedat a concentration of 25 mg/mL of thepureextract(Table2). Incomparedtothetestsoap, a smallerinhibitionzonewasformed inthecontrol soap; 0.7 ± 0.0 cm (Table 4). This might be because ofthenaturalantibacterialpropertyofcoconut oil (Vermén, 2008) used in thesoapbase.

Antibacterial effectivenesstest ofthepreparedadhatodasoap

Thumb impression test was done to test the effectivenessoftheantibacterialadhatodasoapwhich have been already explained. During theexperiment, one hand was washed with the adhatodasoapandtheotheronewascleanedwiththecontrol soap. Now thumbprints of both hands weremadeonasterilisedagarplatetoobservethe growth of microbes in the areas of the thumbprints.It was observed that the number of bacterialcoloniesdeveloped on thethumbprintskeptwithwashed thumbs are lower in number and smallerinsizethanthosegrownonthethumbprints madewithunwashedthumbs. The thumb print made from the hand cleanedwiththeprepared antibacterialsoap did not showbacterial growth in that thumb print area whereas the thumbprint of the handwashedwiththecontrolsoapshowedthepresenceof3-4 smallbacterialcoloniesonit. From the above these thumb impression tests, it can be concludedthattheantibacterialmedicinalsoappreparedduring this study is effective against removingthenormally present bacteriain addition to the specific bacteria thatwere tested in

the laboratory. In this we can say that the prepared adhatoda liquid soap has the promising antibacterial nature.

CONCLUSION

Amongst the all prepared extracts of *Justisia*, methanol soxhlet extract was observed to be the best antibacterial active fraction .When consider the medicinal adhatodasoaps prepared in present study, the antibacterial properties observed in these indicate the promising nature of using *Justicia* extracts in the preparation of value added medicinal soaps. Due to the nowelty approach of this application, this approach is very attractive. As there has not been much published literature so far indicating the use of *Justicia* leaf in medicinal soap products and available published data. Further moreexperimentl and practicals can be carried out to incorporate these bioactive compoundsinto other cosmetic and beauty products as well to produce it at industrial level.

REFERENCES

Chukwulozie PO, ChukwuemekaDE,ChinweOI & Jude ES. Optimization Of A Soap Production Mix Using Response Surface Modeling: A Case Of Niger Bar Soap Manufacturing Industry Onitsha, Anambra State, Nigeria. International journal of scientific and technology research. 2014; 3: 346–352.

De S, Dey YN, Ghosh AK. Phytochemicaal investigation and chromatographic evaluation of the different extracts of tuber of Amorpharphalluspaeoniifolius (Araceae).

International Journal on Pharmaceutical and Biomedical Research. 2010; 1: 150–157.

Driscoll AJ, Bhat N, Karron RA, O'Brien KL & Murdoch DR. Disk Diffusion Bioassays for the Detection of Antibiotic Activity in Body Fluids: Applications for the Pneumonia Etiology Research for Child Health Project.Clinical infectious diseases. 2012; 54:159–164.

EveretteJD, Bryant QM, Green AM, Abbey YA, Wangila GW & WalkerRB.Thorough study of reactivity of various compound classes toward the Folin-Ciocalteu reagent. Journal of agricultural and food chemistry. 2010; 58: 8139–44.

Kaur M, Dhawan P, Damor S, Arora D &Soni IP. Investigating and Exploiting the Antibacterial Potential of Clove (Eugenia caryophyllyum) Extracts while Utilizing it to the Maximum to Develop Liquid Soap against Drug Resistant Bacteria Causing Skin Diseases. International Journal of Pharmaceutical & Biological Archives. 2014; 5: 110–115.

Kole PL, JadhavHR, Thakurdesai P&Nagappa AN. Cosmetics Potential of Herbal Extracts. Natural Product

Radiance. 2005; 4: 315–321.

Namjooyan F, Azemi ME &Rahmanian VR. Investigation of antioxidant activity and total phenolic content of various fractions of aerial parts of Pimpinellabarbata (DC) boiss. Jundishapur Journal of Natural Pharmaceutical Products. 2010; 5: 1–5.

Pa R, Mathew L. Antimicrobial activity of leaf extracts of Justiciaadhatoda L. in comparison with vasicine. Asian Pacific Journal of Tropical Biomedicine. 2012; 2: 1556–1560.

PadmanabhanP & JangleSN.Evaluation of DPPH Radical Scavenging Activity and Reducing Power of Four Selected Medicinal Plants and Their Combinations.

International Journal of Pharmaceutical Sciences and Drug Research. 2012; 4: 143–146.

PRIOR RL,WU X & SCHAICH K.Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry. 2005; 53: 4290–4302.

Sampath KKP, Bhowmik D, Tiwari P &Kharel R, Indian traditional herbs Adhatodavasica and its Medicinal application. J. Chem. Pharm. Res. 2010; 2: 240–245.

Vermen M, Rowell V, Dillague KM, TjunnawanS & Bertha S. NovelAntibacterial and Emollient Effects of Coconut and Virgin Olive Oils in Adult Atopic Dermatitis. Dermatitis. 2008; 19: 308–315.

Wankhede TB. Antioxidant and antimicrobial properties of Adhatodavasica L. Nees. Int. J. of Life Sciences. 2015; 3: 152–156.

WarraAA, Hassan LG, Gunu SY &Jega SA. Cold-Process Synthesis and Properties of Soaps Prepared from Different Triacylglycerol Sources. Nigerian Journal of Basic and Applied Science. 2010; 18: 315–321.

Wijetunge WMANK &Perera BGK. Preparation of Liquid Medicinal Soap Products Using *AdhatodaVasica* (Adhatoda) Leaf Extracts. International Journal Multidisciplinary Studied.2015; 73-81

Wongthongdee N &Inprakhon P.Stability of turmeric constituents in natural soaps. ScienceAsia. 2013; 39: 477–485.

Yeap SK, BehBK, Ali NM, Yusof HM, Ho WY, KohSP, Alitheen NB & Long K. Antistress and antioxidant effects of virgin coconut oil in vivo. Experimental and therapeutic medicine. 2015; 9: 39-42.