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Research Article

STUDIES ON ANTIMICROBAL EVALUATION OF ACHYRANTHES ASPERA, RICE HUSK, TOOTH POWDER AND TOTH PASTE AGAINST ORAL PATHOGENS – GC-MS ANALYSIS

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ABSTRACT

Dental caries is a major health problem throughout the world. For cleaning the toothtooth paste, tooth powder has been used nowadays. But in olden days the people mostly use plantmaterial to get escape from the oral pathogens. *Achyranthes aspera* is one of the medicinal plants. The roots of *Achyranthes aspera* have a tremendous role in preventing the growth of oral pathogens. The aim of the study is to compare the antimicrobial activity including the roots of *Achyranthes aspera*, rice husk, tooth powder and tooth paste. From the result analysis, the roots of *Achyranthes aspera* shows maximum zone of inhibition. GC-MS analysis has been performed to identify the chemical compounds of the root. Some of the compounds have been present in our tooth pastes as the compounds present in root extract of *Achyranthes aspera.*

INTRODUCTION

Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissue of the teeth. Tooth adhering cariogenic bacteria metabolize sugars to produce acid results in demineralization of organic and inorganic portion of the tooth structures. Dental caries is the destruction of enamel, dentin or cementum of teeth due tobacterial activities which it is untreated can cause considerable pain, discomfort and high treatment costs. . (Gautham et al., 2017).

The roots of *Achyranthes aspera* perform tremendous advantages over the teeth. (Abi Beaulah et.al, 2011). It has been widely used as a tooth brush in ancient days. The chewing sticks of the traditional plant possessed antimicrobial properties also cleaned the teeth
mechanically. Their roots have been powdered and used as a tooth powder and the roots itself were used as brushes in India and Terai of Nepal,

The rice husk is a waste product but worth. It is a good antibacterial potential. The rice husk is the outer shell of the grain obtained during the first processing phase of parboiled rice commonly called "husking". Rice husk contains different polyphenols, phenolic acids and vanillic acid derivatives. Some polyphenols are known to have antibacterial. (Gloria et al.,)

One of the oldest methods of cleaning the teeth is using the tooth powder. The abarasive agent of tooth powder includes precipitated calcium carbonate, hydrated alumina, aluminium hydroxide, calcium phosphate dehydrate, alumina trihydrate, magnesium carbonate, etc. (Sarovarreddy et al.,)

Tooth paste plays a significant role for in the morning in everybody's routine life. Toothpaste is a semi-solid drug. It contains sodium, lauryl sulphate, sodium fluoride, Mentha spicata etc, to reduce microbial load. The main purpose of toothpaste is to reduce oral bacterial flora, deliver fluoride to teeth and contribute to dental health.

MATERIALS AND METHODS

The decayed tooth was collected from the nearby dental clinic. To isolate the bacteria from the tooth serial dilution and pour plate technique have been performed.the petriplates were incubated for 24 hrs

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37ºc.

The *Achyranthes aspera* was collected and sorted out. It was powdered and gets dissolved in ethanol. The rice husk was powdered and sieved and gets dissolved in water. 1gm oftooth powder gets dissolved in 10 ml of distilled water separately.

The antibacterial activity was performed Agar plate disc method, Agar plate well cut method and Minimum inhibition concentration

For disc method whatman filter paper are made into 5mm of diameter. Then it was introduced into various sample solution. After an hour, it was placed over the Muller Hinton agarmedium. The petriplates were incubated for 24 hrs. Then the zone of inhibition was observed andmeasured in mm. 1ml of inoculum was poured into petriplate. The wells are cut on the agar plate punctured. . The samples including root extraction of plant, rice husk extract, tooth powder and tooth paste were poured into the punctured well in a respective manner. Then the plates were incubated at 37ºC for 18-24hrs. After 24 hrs the results were observed.

GC-MS ANALYSIS

Gas Chromatography Mass Spectroscopy is used to analyze the chemical components of the plant extract. The plant extract was dissolved in ethanol. The interface temperature was 280ºC and the scan range 40-450 atomic mass units. The oven temperature as initially held at 70ºC for 2 min and then programmed 70-290ºC at 10ºC/min where it was held constant for 5min. Helium was used as carrier gas injected at a constant flow rate of 1mL/min. The total run time was 40 min. The injection volume was 1mL. The solvent delay was 2 min and injected in a split ration of 1:10. Peak analysis was performed by NIST library. The activities of the chemical compounds get analyzed through way 2 drug website.

RESULT:

The zone of inhibition by disc method was shown in the Table. 1.1 In Disc method, the samples reveal a significant antimicrobial activity against the isolated oral pathogens. The highest antibacterial activity was observed in the root extract of *Achyrathes aspera.* The lowest antibacterial activity was seen in tooth powder. The agar well diffusion method was shown in the table. 1.2. This method also reveals the significant antibacterial activity against the isolated oral pathogens. The root of Achyranthes aspera sample shows maximum antibacterial activity.

The Minimum inhibition concentration was shown in table.1.3. The concentration of the sample plays a key role against the inhibition of isolated oral pathogens. At specific concentration the zone of inhibition was highest. This concentration is called the optimal concentration. The optimal concentration of root extract, rice husk, tooth paste and tooth powder are 150µl, 100µl, 100µl, 200µl respectively.

Table 4.1 the value of zone of inhibition by disc method

S.NO	Sample	Solvent	Culture / Zone of inhibition (mm)		
			AJ1	AJ2	AJ3
1	Root extract	Ethanol	16	13	12
2.	Rice husk	Water	8	7	6
3.	Tooth powder	Water	7	6	7

4.	Tooth paste	Water	11	11	9

Table: 4.2 Thevalue of zone of inhibition by Agar well cut method

S.No.	Sample	Solvent	Culture/ Zone of inhibition (mm)		
			AJ1	AJ2	AJ3
1.	Root extract	Ethanol	15	10	8
2.	Rice husk	Water	9	8	8
3.	Tooth powder	Water	9	8	7
4.	Tooth paste	Water	7	6	7

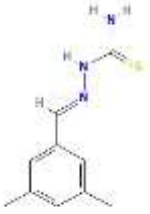
Table: 4.3 The value of inhibition by Minimum inhibition concentration

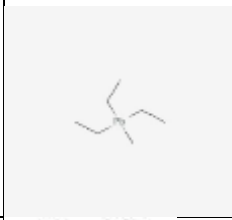
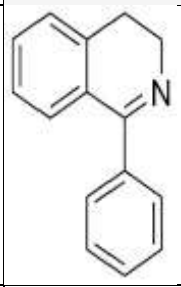
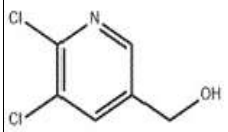
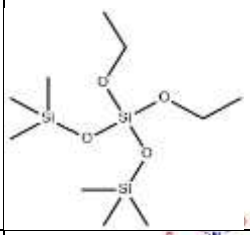
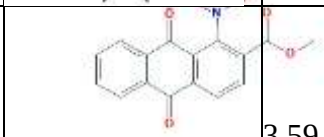
Sample	Solvent	Culture/ Zone of inhibition (mm)											
		AJ1 (µl)				AJ2(µl)				AJ3(µl)			
		50	100	150	200	50	100	150	200	50	100	150	200
Root extract	Ethanol	14	15	16	15	9	10	11	11	7	8	9	8
Rice husk	Water	12	13	11	10	7	8	7	6	5	6	6	5
Tooth powder	Water	12	14	12	11	7	6	5	5	6	7	6	5
Tooth paste	Water	12	13	13	14	9	10	10	11	5	5	7	8

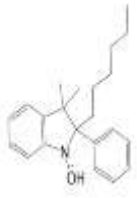
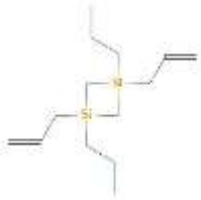
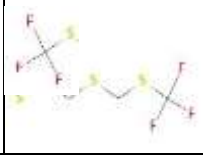
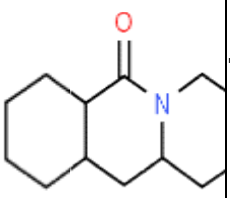
Plate 4.2 shows the zone of inhibition by Minimum inhibition concentration

Plate 1.1 the zone of inhibition by disc method

Table 4.4: Bioactive compounds of root extract of *Achyranthes aspera*

Sl. No	Retention time	Name of the compound	Molecular formula	Molecular weight (g/mol)	Structure	Peak area (%)
1.	8.668	2 Hydroxy1 isoindolinone	$C_{12}H_{15}NO_2$	433.5		1.67
2.	14.123	14 Pentadecenoic acid	$C_{15}H_{28}O_2$	240.38		3.63
3.	15.258	5-Bromovaleric acid, pentadecylester	$C_{20}H_{39}BrO_2$	391.4		3.85
4.	15.258	3,5, Dimethylbenzaldehyde thiocarbamoylhydraone	$C_{10}H_{13}N_3S$	240.8		3.60

5.	16.535	2- Chloro 4,6-di-piperidinl-yl-1,3,5, triazine	$C_{15}H_{10}CIN_3$	28.78			2.41
6.	16.39	Plumbale triethyl methyl		$C_7H_{18}Pb$	309.0		0.94
7.	20.799	4 Phenyl3,4, dihydroisoquinoline		$C_{15}H_{13}N$	296.5		3.59
8.	16.393	2- Pyridinemethanol, 3,5, dichloro 6-methyl		$C_6H_6C_{12}N_2$	240.8		3.60
9.	17.773	Silicic acid, diethyl bi(trimethylsilyl) ester		$C_{10}H_{28}O_4Si_3$	296.5		3.59
10.	17.77	1-Nitro-9,10-dioxo-9,10-dihydroanthracene-2 carboxlic acid diethylamide		$C_{16}H_9N0_6$	311.24		3.59

11.	20.799	Indole 2 -one-2,3 dihydro-N-Hydoxy-4-methozy 3-3-dimethyl	$C_{13}H_{13}NO_3$	207.23		1.67
12.	20.799	1,1,3,3, tetraallyl 1,3-disilacyclobutane	$C_{14}H_{24}Si_3$	248.51		1.67
13.	17.660	2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	$C_{17}H_{14}O_4$	282.29		3.09
14.	20.856	7,7,9,9,11,11,-Hexamethyl 3,6,8,10,12,15 Hexa7,9,11, trisilaheptatene	$C_{14}H_{36}O_6Si_3$	384.69		6.17
15	17.565	Ethane,1-(4,4,4- trifluoro-1,3- dithiobutyl)-2-(3,3,3,-trifluoro-1,2- dithiopropyl)	$C_5H_6F_6S_4$	308.4		6.94
16.	20.544	Dodecahydropyridol (1,2-b)isoquinone	$C_{13}H_{21}NO$	287.4		7.33
17	8.668	2-Acetylbenzoic acid	$C_9H_8O_3$	164.16		1.67

Activity of Bioactive compounds by ethanol extract of ***Achyranthes aspera*** **by GC-MS analysis**

S.No.	Name of the Compounds	Activities
1.	2 Hydroxy 1 isoindolinone	Antibacterialactivity, antiinfective activity
2.	14 pentadecenoic acid	Antibacterial activity, antiviral activity, Colony inhibition factor.
3.	5 Bromovaleric acid, pentadecyl ester	Antifungal,Calcium channel activator, Antiinfective
4.	3,5, Dimethylbenzaldehyde thiocaramoylhydraone	Antiviral, Whitening activity
5.	2-Chloro 4,6-di-Piperidinl-yl-1,3,5, triazine	Antibacterial, Antiprotozoa, chitinase inhibitor cellulose inhibitor.
6.	Plumbale triethyl methyl	Transcripition fator rho inhibitor, Antibacterial
7.	4 phenyl 3,4, dihydro isoquinoline	Antiviral, presence of whitening factor
8.	2- Pyridinemethanol ,3,5, dichoro 6-methyl	Antianemic, antiviral, act as an antidote
9.	Silicic acid, diethyl bi(tirmethylsilyl)ester	Cell wall Synthesis inhibitor, antibacterial
10.	Dodecahydropyridol (1,2-b) isoquiinone	Antibacterial, antiviral
11.	Indole 2 -one-2,3 dihydro-N-Hydoxy-4-methozy 3-3-dimethyl	Nuclease inhibitor
12.	2-Acetylbenzoic acid	Antiviral, antibacterial
13.	1-Niro-9,10-dioxo-9,10- dihydroanthracene-2 carboxlic acid diethylamide	Antibacterial activity
14.	1,1,3,3, tetraallyl 1,3-disilacyclobutane	Mucomembranous protector, Antiinfective
15.	2-(Acetoxymethyl)-3-(methoxycarbonyl) biphenylene	Cell adhesion molecule inhibitor
16.	7,7,9,9,11,11,-Hexamethyl 3,6,8,10,12,15 Hexa7,9,11,trisilaheptadecane	Antiviral, antibacterial
17.	Ethane,1-(4,4,4- trifluoro-1,3- dithiobutyl)-2-(3,3,3,- trifluoro-1,2- dithiopropyl)	Acidifying agent, chitinase inhibitor

DISCUSSION

Medicinal plant is the most exclusive source of life saving drugs for majority ofworld's population. They continue to be an important therapeutic acid for alleviating the ailments
of human kinds. Plants have been used as medicines by all cultures from ancient times to the recent days. The information and benefits of herbal drugs are exists in our ancient literature of Ayurvedic and Unani medicine. Medicinal plant plays an important role in human health care since ancient times. (Abi Beulah.,et al., 2011)

According to Roma yodav 2016, the zone of inhibition for the root extracts of *Achyranthes aspera* was 12mm. The average value for the zone of inhibition is 13mm in accordance to this report. The

plant extract was found to be effective at a higher concentration. A study conducted by Giri et al. 2015, showed 28±1.4 zone of inhibition at 100% concentration. From this analysis,the concentration 150µl is suitable for the maximum antibacterial activity.

From the root extract of *Achyranthes aspera* 57 compounds were identified. Among the chemical compounds 2 Hydroxy 1 isoindolione, 14-Pentadecenoic acid, 2-Chloro 4,6-di Piperidinl-yl-1,3,5, triazine, Plumbale triethylmethyl, Silicic acid, diethyl bi(trimethyl silyl)ester,Dodecahydropyridol (1,2-B)isoquionone 2-Acetylbenzoic acid, 1-Nitro-9,10-diozo-9,10- dhydroanthracene-2 carboxlic acid diethylamide, 7,7,9,9,11,11,-Hexamethyl 3,6,8,10,12,15, Hexa 7,9,11,trisilaheptadecane have specific activity against cariogenic organisms.

The conducted computational study reveals the bioavailability properties such as absorption, distribution, and metabolism is present in *Achyranthes aspera* as a phytochemicals. The obtained results indicated that the *A.aspera* has number of bioactive compounds interacting efficiently with biofilm synthesis and mediating the enzyme of cariogenic bacteria thereby preventing the expression of cariogenic virulence. (Murugan et al., 2013)

Muriady et al. 2022, reveals that the rice husk shows the zone of inhibition at 6.53mm.In the present study notifies that the zone of inhibition was observed 7mm. Based on the zone of inhibition for the rice husk the antimicrobial power strength were categorized as very weak (<5mm), intermediate (5-10mm), strong (10-20mm). (Muriady et al,.2022) the rice husk shows the intermediate values of antimicrobial activity in accordance with the above suggestions.

Sarvoor reddy et.al worked to proclaim the antimicrobial activity of tooth powder and demonstrated the zone of inhibition about 9.5mm. This study tells that the zone of inhibition wasfound to be 7mm. Tiswari et.al, worked over the antimicrobial activity of Herbal tooth powder. They conclude the zone of inhibition was found to be 13mm.

Abdirahman Mohammed (2024) studies about the antimicrobial activity of tooth paste. He declared that the maximum zone of inhibition was found in 150µl concentration. This study suggests that at 200µl concentration is suitable for the maximum zone of inhibition as about 14mm. Ayesha parveen et.al, 2017 concentrated over the antibacterial activity of herbal tooth paste and reported that the zone of inhibition was found to be 18mm.

From the above all suggestion, the medicinal plants possess most of the chemical compounds, so it opposes the growth of microorganism even in an optimal condition. Even though the chemicals compounds are present in the tooth powder and tooth paste, it shows less antimicrobial activity as compare to plant material. Plant materials are recommended to use as a tooth cleaning agent on behalf of tooth paste.

The tooth paste consists of plant materials in the form of chemical composition. The herbal composition of tooth paste includes *Piper longum* (long pepper), *Zanthozylum armatum* (rattan pepper), *Syzygium aromaticum* (clove) and *Mentha spicata* (mint). The phytochemical analysis of Piper longum by Maitreyi et al.,2010 reveals the presence of 2, Chloro 4,6-di- Piperidinyl 1,3,5 triazine. According to Mahmoud et al.,2007 illustrated the presence of hexadecanoic acid, 1-triethyl ethyl ester in *Syzygium aromaticum* (clove). The phytochemical analysis of *Zanthoxylum armatum* (rattan pepper) demonstrated the presence of Hexadecanoic acid by Prasanta et al.,2011 Jain et al 2016 conducted an experiment to analysis the phytochemicals present in *Mentha spicata* and shows the presence of pentadecanoic acid in the extract.

The above all chemical compounds perform antibacterial activity as mentioned in the table4.6. Because of the reason the tooth paste contains such products. *Achyranthes aspera* naturally posses those compounds and had been used as tooth brush in olden days.

This report concludes that the naturally available plant materials are advisable in daily practices. It helps in reducing the side effects. It maintains the oral hygienic. In the fast growing world, the use of readily available plant material is difficult to practice. But to overcome the demand along with the easy use herbal products are conveniently recommended. Herbal productsalso seem to have potent antimicrobial activity.

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