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Original Research Article

ANTIPYORRHOEAL ACTIVITY OF POLYHERBAL DRUGS USING FERMENTATION TECHNIQUE

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ABSTRACT

In this study, the extract obtained from the bark of Guava, Jack plum and Toddy palm was studied. The bark was obtained from BIT Mesra campus, Jharkhand. The bark was obtained in the wet state and no epiphyseal growth was observed. The bark was dried in sunlight for 2 weeks and moisture was reduced. Guava bark coiled on drying. The dry state bark was powdered and extraction process (cold extraction) was performed for the presence of phytoconstituents. All the powdered drugs were mixed (1:1:0.3) and subjected to fermentation. Fermented product was prepared and analyzed for its physical, chemical and pharmacognostical studies.

Keywords: Polyherbal drugs, Fermentation, Phytochemical test, Analytical specification, Organoleptic characteristics.

INTRODUCTION

Pursuits of health and longevity have been the main preoccupations of man in all ages, but the methods have varied from age to age. This quest for health has led man to investigate every existent article under the sun from a therapeutic point of view. But during such investigations whatever drug our ancestors found could be administered to the patients as they were. To make them palatable and to preserve them for a longer time, they had to make some modifications. Among such dosage forms, fermentative preparations gained the highest therapeutic and pharmaceutical values. Fermentative preparations contain self generated alcohol which in turn act as a preservative and helps in the quick absorption, increases the bioavailability and pharmacological activity.

In the present work the whole bark of *Psidium guajava* L. (Guava), *Syzygium cumini* L. (Jamun), *Borassus flabellifer* L. (Toddy palm) were collected, dried and powdered and the final product was obtained using fermentation technique. Antipyorrhoeal activity of the fermentative product was claimed as per the Traditional system of Medicine. The extract of guava, jamun and toddy palm bark is a folklore system of medicine (comprises medical aspects) of traditional knowledge that developed over generations within various societies before the era of modern medicines. In this extract preparation, the message was transferred from one generation to next generation verbally. Family members used it as a mouthwash and got relief from sore gum.

MATERIALS AND METHODS

Materials

Jamun bark powder: 80g

Toddy palm bark powder: 250g

Guava bark powder: 80g

Honey: 250g

Jaggery: 250g

Water :2500ml

Method (Cold Extraction)

The barks were dried in sunlight for 2 weeks till the moisture evaporated. Dried barks were crushed into powder. 3 litre conical flask was sterilized using smoke of neem (*Azadirachta indica*) and pippali (*Piper longum*). 2500 ml of distilled water was taken in a flask. Jaggery was crushed and dissolved in the water. Honey (250g) was added to it. 80 g of each powder was added and mixed properly. The mixture was then left for fermentation. After certain intervals fermentation test was performed.

Table1: Fermentation Test

S. No.	Duration of tests	Fermentation Test			
		Foaming	Taste	Candle Enlightenment	Lime water
01	1 day from preparation	Appeared	Slightly sour	Extinguish	Precipitate formed
02	2 day from preparation	Appeared	Slightly sour	Extinguish	Precipitate formed
03	5 day from preparation	Appeared	Sour	Extinguish	Precipitate formed
04	7 day from preparation	Appeared	Sour	Extinguish	Precipitate formed
05	9 day from preparation	Appeared	Very sour	Extinguish	Precipitation formed
06	13 day from preparation	Slight appearance	Very sour	Enlightened	Less precipitate formed
07	15 day from preparation	Decreased	Very sour	Enlightened	Less precipitate formed
08	16 day from preparation	Decreased	Very sour	Enlightened	No precipitation
09	21 day from preparation	No foaming	Very sour	Enlightened	No precipitation

The above tests ensured the completion of fermentation process. Once the fermentation process was complete, filtration was done using whatman filter paper. Filtrate was then subjected for further studies.

Thin Layer Chromatography

Extract (suitably filtered) was spotted on silica gel TLC plates developed in solvent systems of different ratio

of butanol, acetic acid and water. The ratio at which detection occurred was 3:1:5 (Butanol, acetic acid, water). The plates were dried at room temperature and sprayed with ninhydrin solution.

Phytochemicals Test

The extract was acidified using diluted HCl or H₂SO₄ (few drops) for the tests.

Alkaloids:

Tests	Reagents
1.Mayer Test	HgCl ₂ +acidified solution+KI
2.Dragendroff Test	Bi(NO ₃) ₃ .H ₂ O(Bismuth Subnitrate) +KI + acidified solution
3.Wagner Test	I ₂ +KI+acidified solution
4.Hager Test	Picric acid+acidified solution

Flavonoids:

Test	Reagents
Sulphuric acid test	Conc. H ₂ SO ₄ +few drops of extract

Polyphenols:

Test	Reagents
Ferric chloride test	FeCl ₃ + H ₂ O + few drops of extract

Tannic Acid

Test	Reagents
Tannic acid test	Powder + Tannic acid

Saponins

Saponin content was detected by formation of foam on shaking, which persisted for a minimum of 15 minutes.

Alcohol Content

A proper distillation setup was made. 25 ml of preparation (extract) being examined was measured accurately at 24.9⁰ C to 25.1⁰C and transferred to the distillation flask. It was diluted with 150 ml of water and little pumice powder. Distillation head and condenser were attached. Distillation was done and not less than 90 ml of distillate was collected. The temperature was adjusted to 24.9⁰C to 25.1⁰C and distillate was diluted to 100 ml. Relative density was calculated at room temperature. According to the table of relation between relative density and ethanol content in” Laboratory guide for the analysis of Ayurveda and Siddha Formulation”, ethanol content was determined.

Saponification Value

To determine the saponification value of the extract, 0.5 g of the sample was dissolved in 12.5 ml of 0.5N alcoholic KOH solution. The mixture was incubated in boiling water bath for 30 minutes which was then cooled at room temperature and titrated with 0.5N HCl, using 1% phenolphthalein indicator. Besides, a blank was also allowed to run to have a precise comparison among duplicates and the mean result was considered.

Reducing Sugar and Non-Reducing Sugar

Reducing Sugar- The solution was diluted in water and warmed until fully dissolved. Fehling's solution was added while stirring. Non-Reducing Sugar-Benedict's test was performed.

Test for Heavy Metals (Lead, Mercury, Arsenic, Cadmium)

The extract was treated in Microwave Digester and then further testing was done through ICPOES

(Inductively Coupled Plasma Optical Emission Spectroscopy).

Microscopy

The powders were observed under the microscope after preparing slides using powdered bark, chloral hydrate and Iodine. Lignification detection was done using phloroglucinol and dil. HCl.

Microbial Contamination

Nutrient media was prepared. 3 test tubes of control were prepared.

1. Positive control - 5ml media+10 µl of S.aureus
2. Negative control- 5ml media
3. Test - 4ml media+1 ml %v/v extract

After 1 day of incubation, the turbidity of the test tubes was observed and comparison was done

RESULTS AND DISCUSSION

Organoleptic Characters of Bark (Wet State)

Characters	Guava	Jack Plum	Toddy Palm
Appearance	Smooth surface, moisture present	Woody rough surface, high moisture present, fungal growth	Woody fibrous surface, Moisture present
Colour	OS-light brown IS-light brown	OS-greenish brown IS-deep red	OS-Dark brown IS-light brown
Taste	Sweet musky	Mild sour, Astringent flavour	No characteristic Taste
Odour	Slightly sweet	Sweet, astringent	No characteristic odour.
Texture	Longitudinal	Longitudinal	Longitudinal

Organoleptic Characters of Bark (Dry State)

Characters	Guava	Jack Plum	Toddy Palm
Appearance	Smooth straight	Woody rough surface, fungal growth which vanished after drying	Highly fibrous
Colour	OS-Dark brown mud color IS-Light brown	OS-Dark brown IS-Light copper color	OS-Dark brown IS-Yellow
Odour	Dry Resinous smell	Slightly sweet, astringent smell	No characteristic odour
Texture	Longitudinal	Longitudinal	Longitudinal
Shape of Bark	Curved	Curved	Flat

OS-Outer Surface, IS-Inner Surface

Organoleptic Characters of Bark (Powdered Form)

Characters	Guava	Jack Plum	Toddy Palm
Colour	Very light brown / greyish appearance	Brownish appearance	Light brown appearance
Taste	Slightly sour	Woody sour taste	Woody taste
Odour	Resinous smell	Astringent smell	No characteristic smell

Phytochemicals Test

Trace amount of alkaloids were present.

Polyphenols were present.

Flavonoids and saponins were absent.

Jamun didn't contain tannic acid while guava and toddy palm bark contained tannic acid.

Saponification Value

Saponification value of the extract was obtained to be 78.568 mg KOH

Analytical Specification of Fermented Product**1. Description**

A. Colour- Dark brown (wine colour)

B. Odour- Sour odour

C. Consistency- Consistent liquid (no gritty particle). Dark brown colored slit deposition at the bottom of the container.

D. Boiling Point- the Boiling point of the solution was obtained to be 89°C.

Specific Gravity at 25°C

Specific gravity was found to be 0.9733.

Alcohol Content

Alcohol content (ethanol) was obtained to be 21%.

Reducing Sugar

The solution began to show color change due to the formation of red coloured precipitate hence resulting in presence of reducing sugar.

Non-Reducing Sugar

With Benedict's test for non-reducing sugar, greenish precipitate was formed which confirmed the presence of trace amount of non-reducing sugar.

Thin Layer Chromatography

2 spots appeared

R_f (Retention factor) was obtained for each spot,

R_f 1 = 0.66

R_f 2 = 0.6

Total Acidity

pH of the extract was found out to be 4. It was again checked after an interval of 1 week, the result being the same.

Test for Heavy Metals

No heavy metals were detected.

Table: Heavy metal detection through ICP-OES

Sample ID	Analyte	Mean (mg/L)
Std 5ppm		
As	193.696	5.056
Ca	422.673	4.856
Cd	226.502	5.058
Cr	205.560	5.025
Fe	259.939	5.073
Mg	279.077	5.081
Mn	257.610	5.031
Pb	220.353	5.084
Zn	213.857	4.988
SAMPLE		
As	193.696	-0.092
Cd	226.502	-0.087
Pb	220.353	-0.032
SAMPLE		
2ml	193.696	-0.093
As	226.502	-0.088
Cd	220.353	-0.031
Pb		

Microbial Contamination

Turbidity appeared in the solution containing 4ml media and 10ml 1% v/v extract and 10 μ L of *S.aureus*. Therefore it can be concluded that microbial contamination was present in the extract.

Microscopy

A. Guava Bark Powder

The powder had light brown to greyish appearance, slightly sour taste and resinous odour. Under the microscope, numerous resins and abundant yellowish brown masses characterized the slide. Circular and rectangular stone cells were also observed.

B. Jack Plum Bark Powder

The powder had brownish appearance, woody sour taste and astringent odour. Under the microscope, resins, clustrous Ca-oxalate and brown matter were observed.

C. Toddy Palm Bark Powder

The powder had light brown appearance, woody taste and no characteristic odour. Under the microscope, large rectangular stone cells, resins and starch grains were observed.



Fig A: Powder microscopy of guava

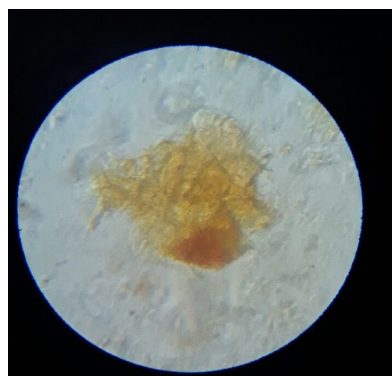


Fig B: Powder microscopy of jack plum



Fig C: Powder microscopy of toddy palm

CONCLUSION

Traditionally all the plants mentioned in the text are used to clean the teeth and other related problems to maintain good health of the teeth. The author has tried to prepare the fermented product of these plants/parts, their correct identification and physical constant values were analyzed using some modern techniques. The preparation was applied on decaying of the tooth and was found that the pyorrhoea present all over the teeth gets cleaned using 2-3 times per day for 1 month.

FUTURE SCOPE

- I. The preparation should be subjected to pharmacological experiment for confirmation.
- II. The chemistry of all the ingredients should be carried out using modern analytical techniques.

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