



GC-MS analysis, antimicrobial, antioxidant activity of an Ayurvedic medicine, *Salmali Nirayasa*

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ABSTRACT

With the increasing acceptance of herbal medicine as an alternative form of healthcare the screening of herbal medicines for active compounds has become very interesting as they may serve as promising sources with novel mechanism of drug action. The medicinal usage of *Salmali*, *Bombax cieba* has been reported in the traditional systems of medicine such as Ayurveda and Siddha. Current study is to find out the antioxidant properties, antimicrobial activities, GC MS analysis of the drug and the TLC ratios. Antioxidant competence of the drug was assessed by reducing power assay, peroxidase assay and catalase assay. The antimicrobial activity study of this drug on *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E.coli* and *Candida albicans* showed fare degree of inhibition where as on *Candida albicans* it was quite effective. The GC MS analysis indicated the presence of Cinnamic acid, *m*-derivative (RT value – 39.548), Benzimidazole-3-derivative (RT value- 39.548), Pyrazole (RT value – 39.258), Metolachlor (RT values – 38.963), Triamterene (RT Value -38.963) and Nitrazepam (38.963) among other minor compounds. The presence of these bioactive molecules in *Salmali* resin strongly advocate its strong antimicrobial, antioxidant activity as well for other medicinal values as claimed in Ayurveda.

Key words: Ayurveda, *Salmali*, TLC, GC MS analysis, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, *E.coli*, Cinnamic acid, Nitrazepam

INTRODUCTION

Bombax ceiba or *Bombax malabaricum* is a deciduous tree commonly known as Silk cotton tree or Indian Red Kapok belonging to family Bombacaceae. This tree is known as Rakhta salmali in Indian system of Ayurveda. Recently the plant has undergone extensive scientific research for its medicinal value. In Ayurvedic medical practice *Salmali nirayasa* (Resin) is used for the treatment of acute dysentery, haemoptysis of pulmonary tuberculosis, influenza and menorrhagia. It is used in bladder disorders, calculus, catarrh, cystitis, gonorrhoea, sores and wounds. [1,2] It is reported to have following properties like stimulant, expectorant, astringent, haemostatic, anti-inflammatory, aphrodisiac, diuretic, antidiarrhoeal, cardiogenic, emetic, demulcent, anti dysenteric and antipyretic have been mentioned.[3, 4, 5] The pharmacological activity of *Bombax ceiba* as anti inflammatory in vitro was described by Ananda Rajagopal *et al* (2013). [6] Due to presence of active flavanoids and lupeol the extract of stem bark of *Bombax ceiba* has significant anti obesity potential against HFB induced experimental obesity due to modulation in FAS and PTP-TB signaling in Wistar rats.[7] The presence of triterpenoid compound account for hypoglycemic activity of *Bombax ceiba* in the management of diabetes [8]

Shamimin and c-flavonil glucoside from *B. ceiba* leaves showed to have hypotensive activity and methanolic extract of flowers of *B. ceiba* possess a hepato-protective activity. The stem bark of *B. ceiba* exhibits a significant anti angiogenic activity. [9, 10] The *Bombax* plant or its parts when used singly or in combination with other herb also possess medicinal uses.

The gymnasium part of the flower was reported to have antioxidant and RBC membrane stabilization properties. [11] The antioxidant and immune-modulatory activity of the bark of this plant has been reported by Wahab *et al*, 2014.[12] Free radical scavenging property of *Bombax ceiba* root extracts was reported by Jain *et al*, 2011.[13] Antioxidant and anti hemolytic activities of *Bombax ceiba* spike and fruit extracts was reported by Divya *et al*, 2012.[14]

The present study is to understand the efficacy of Salmali as a drug. The GC MS analysis revealed the presence of Cinnamic acid derivatives, Benzimidazole-3-derivative, Metolachlor, Nitrazepam and Triamterene, which are proven biomolecules used as medicines. The claims of medicinal efficacy of Salmali in treating various diseases as reported in ayurvedic literature, correlates well with the presence of these biomolecules in the niryasa of Salmali.

In the present study, antioxidant activity, antimicrobial effect, GC MS analysis and determination of TLC ratio of the popularly known herbal medicine, Salmali niryasa (Resin of *Bombax ceiba*) was undertaken.

EXPERIMENTAL SECTION

COLLECTION OF SAMPLES

Medicine was purchased from standard Auyurvedic shop in Chennai.

COLLECTION OF MICROORGANISM

Cultures of micro organisms collected for antimicrobial sensitivity test are

- *Klebsiella pneumoniae*
- *Staphylococcus aureus*
- *Escherichia coli*
- *Candida albicans*

The above microorganisms were collected from King Institute of preventive medicine and Research, Chennai. Samples were collected in slants and were sub cultured for antimicrobial tests.

Sub culturing of microorganism for the anti microbial test

Media used for sub culturing of microorganism: For *Klebsiella pneumoniae* - Simon citrate; for *Staphylococcus aureus*- Blood Agar; for *Escherichia coli* - Nutrient Agar and for *Candida albicans* -YEPD was used.

ANTIMICROBIAL ACTIVITY TEST

The assay was carried out according to the method of Natarajan *et al*, (2005) with some modifications.[15]

Preparation of sample

About 500mg of drug were weighed and dissolved in 100ml of distilled water and filtered. The filtrate was used to test the efficacy of antimicrobial activity.

Test Organisms

Gram positive & Gram negative bacteria were used as test organisms for this study. Gram positive bacteria such as *Staphylococcus aureus*, Gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia* and fungus like *Candida ablicans* were tested. The organisms were sub cultured on to agar plate in order to determine their viability. Stock cultures were maintained on agar slants at 4 °C and then sub-cultured in agar plates at 37 °C prior to each antimicrobial test.

Activity by agar well diffusion assay

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti bacterial and anti-fungal activities of the plant samples. Corresponding Agar (Hi-media) for bacteria and for fungus were prepared according to the manufacturer's instructions. The antibacterial activity of drug was determined by agar well diffusion method. Agar in the Petri plate after solidification was inoculated with the test microorganisms, by

spreading the bacterial inoculums under aseptic conditions. Wells of 5mm diameter were punched in the agar medium with sterile cork borer and filled with drug in the particular concentration. The antibiotic, namely, penicillin was used in the test system as positive control. The plates were incubated at 37 °C for 24 hrs. The antibacterial activities were assessed by measuring the diameter of the zone of inhibition for the drug and antibiotic.

PREPARATION OF TLC PLATES

TLC of the prepared extract of the Salmali was undertaken and spots were identified in the solvent systems with the following ratio: Hexane: Ethyl acetate for Phytosterols and Acetone: Ethyl acetate, for Alkaloid molecules based on the method employed in the literature. [16, 17] 30g of silica gel was weighed and homogenous suspension with 60ml distilled water for two minutes was prepared. The suspension was distributed over the plate which was air dried until the transparency of the layer disappeared. The plates were dried in hot air oven at 110°C for 30 min and then stored in a dry atmosphere and used whenever required.

Prior to TLC, the samples were dissolved in a suitable solvent and then applied to the origins of a TLC plate.

Formula for Calculating R_f value: $R_f = (\text{Distance travelled by the solute}/\text{Distance travelled by the solvent}) \times 100$.

Application of the Substance Mixture for separation: The solutions of the different samples were taken in capillary tubes and were spotted on a TLC plate 2cm above its bottom.

Development of the chromatogram: After application of the sample on the adsorbent, the TLC plate was kept in the solvent in TLC glass chamber and the mobile phase was allowed to move through adsorbent phase up to 3/4th of the plate. The separation took place and the colored spots were obtained. The R_f values for different spots for different extracts were determined and results have been tabulated in Table 3.

ANTIOXIDANT TEST

Preparation of sample

1gm of sample were weighed and dissolved in 10ml of distilled water and filtered. The concentration of drug was varied from 0.2 to 1ml.

Determination of Reducing Property

The reducing power of the herbal medicine extract was determined by a slightly modified method of (Oyaizu,1986).[18]The reducing ability of the drug extract was measured by the transformation of Fe³⁺ to Fe²⁺ in the presence of the extract at 700nm. Increased absorbance of the reaction mixture indicates increased reducing power. 1 ml of each plant extract concentration (0.1, 0.5 and 1 mg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and Potassium ferricyanide [K₃Fe(CN)₆] (2.5 ml, 1 %). The mixtures were then incubated at 50°C for 20 min. Aliquots (2.5 ml) of Trichloroacetic acid (10 %) were added to each mixture, which were then centrifuged for 10 min at 1000 rpm. The upper layer of the solutions (2.5 ml) were mixed separately with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1 %), and the absorbance levels were measured at 700 nm using a spectrophotometer.

Guaiacol Peroxidase (POD)

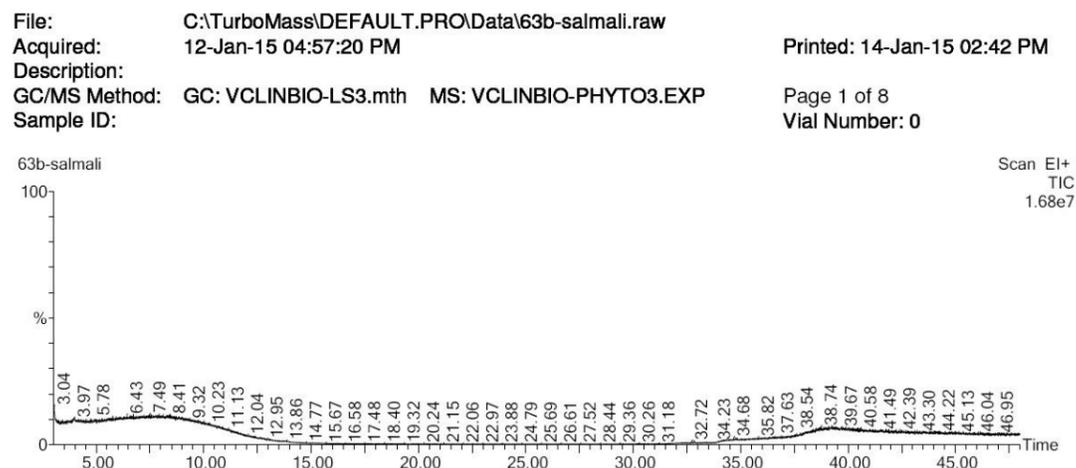
Peroxidase activity was determined according to Panda *et al*, (2003) with slight modification.[19] Each solution were treated with 2ml of a solution containing Guaiacol, H₂O₂ and phosphate buffer (pH 7) in the concentrations of 1%, 40mM and 100mM, respectively. The enzyme produced a colourful product by using H₂O₂ and Guaiacol as substrates. The absorbance of the product was monitored at 470 nm and peroxidase activity was indicated in the form of graph.

Catalyse (CAT)

Catalase activity was determined according to (Aebi and Lester (1984). [20] The decomposition of H₂O₂ was followed as a decrease in absorbance at 240 nm in a UV/Vis spectrophotometer. 50 mM potassium phosphate buffer, pH 7.0 and 10mM H₂O₂ mixture was used. The extinction coefficient of H₂O₂ (40 mM⁻¹ cm⁻¹ at 240 nm) was used to calculate the enzyme activity that was expressed in terms of milli moles of H₂O₂ per minute per gram fresh weight.

RESULTS AND DISCUSSION

Figure 1. The GC-MS patterns of Salmali resin

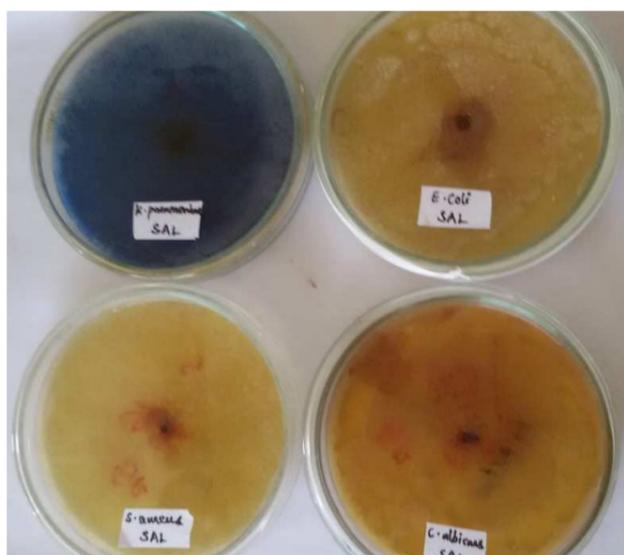


The GC MS analysis indicated the presence of Cinnamic acid-m-derivative (RT value-39.548), Benzimidazole-3-derivative (RT value-39.548), Pyrazole (RT value-39.258), Metolachlor (RT value-38.963), Triamterene (RT Value-38.963) and Nitrazepam (RT Value- 38.963) among other minor compounds as depicted in Table 1 and Figure 1.

Table 1. GC-MS results indicating the names of major compounds, Retention values and molecular formula

Sl. No.	Phytoconstituent	Retention Time (In Min)	Structure	Library
1.	Benzimidazole-3-derivative	39.548	$C_{15}H_{12}N_2O_2$	Main_lib
2.	Cinnamic acid, m- derivative	39.548	$C_{13}H_{18}O_3Si$	Main_lib
3.	Pyrazole-3-derivative	39.58	$C_5H_5IN_2O_2$	Main_lib
4.	Metolachlor	38.963	$C_{15}H_{22}ClNO_2$	Nist_msms
5.	Nitrazepam	38.963	$C_{15}H_{11}N_3O_3$	Nist_msms
6.	Triamterene	38.963	$C_{12}H_{11}N_7$	Replib

The antimicrobial effect on various microorganisms using *Salmali* is indicated in Table 2, Figure 2 and Figure 3.

Figure 2. Shows the zones of Inhibition by *Salmali* on different microorganisms

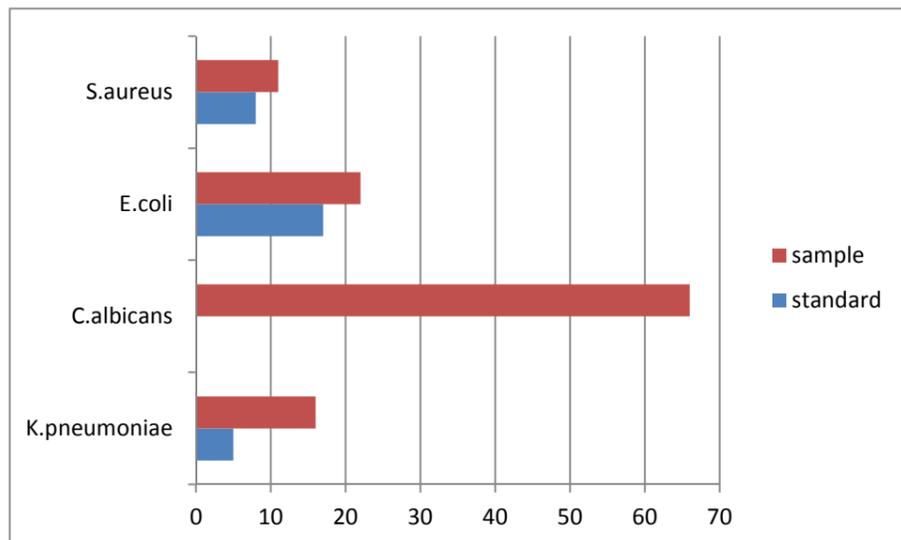


Figure 3. Indicates the percentage inhibition of microorganism by *Salmali*

Table 2. Antimicrobial effect of *Salmali* on different Microorganisms

Concentration of Extract (Mg/ml)	Microorganisms	Zone of Inhibition (mm)	
		Salmali	Penicillin
50	<i>Staphylococcus aureus</i>	6	5
50	<i>Escherichia coli</i>	4	16
50	<i>Candida albicans</i>	24	0
50	<i>Klebsiela pneumoniae</i>	8	8

It was observed that Salmali has antimicrobial potential in general and antifungal activity in particular. The antioxidant activity of Salmali is shown in Figure 4, Figure 5 and Figure 6.

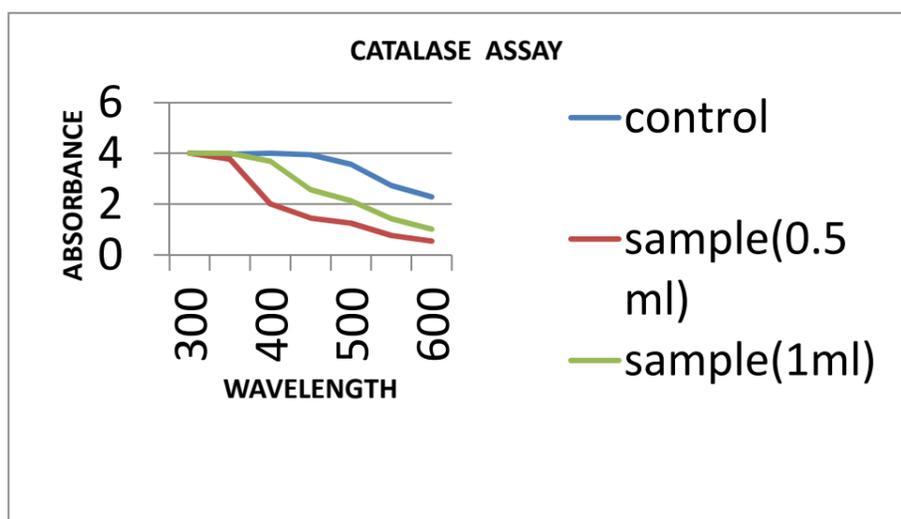


Figure 4. Indicates the Catalase activity of *Salmali*

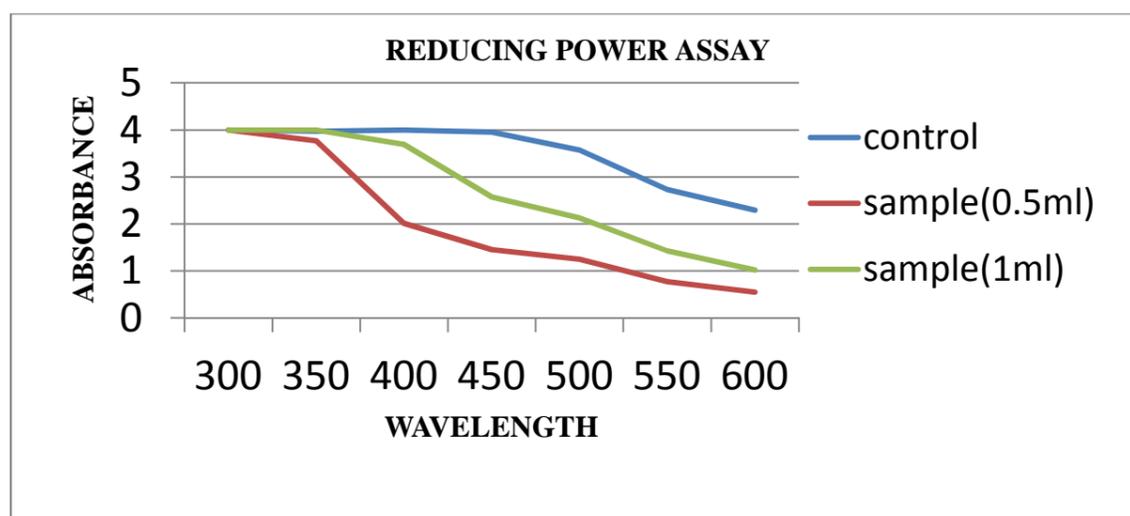


Figure 5. Indicates the reducing power activity of *Salmali*

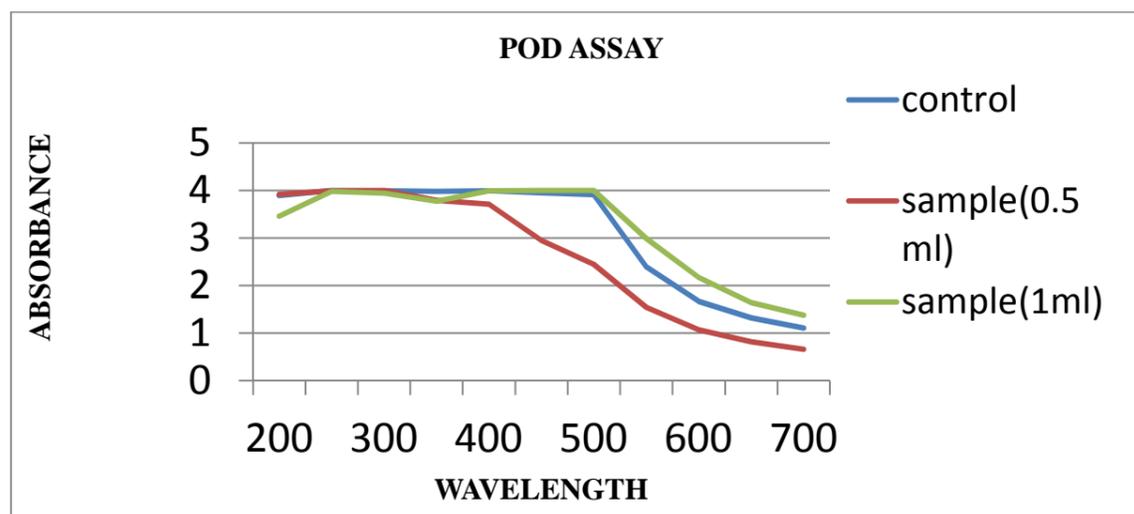


Figure 6. Indicates the POD assay activity of *Salmali*

GC-MS analysis for the sample was carried out in a Shimadzu GC-MS_P 2010 gas chromatograph fitted with DBI (methylphenylsiloxane, 30m x 0.25 mm i.d) capillary column. Carrier gas, helium with a flow rate of 1ml/min initial temperature 50c ramp 10 c/min to 150c, hold 10min ramp 10c/min to 280c hold 10 min injector temperature 200c volume detector temperature 290c volume injected 2 L: split ratio 25:1. The MS operating parameters were as follows: ionization potential 70 eV ion source temperature 180c solvent delay 3.00mm scan speed amu/s and scan range 30-600 amu eV voltage volts.

The concentrated extract form of the drug is injected into the GC-MS instrument (Hewlett Packard 5890 GC/MS with mass selective detector with turbo mass gold perkin elmer. With increasing the temperature the sample is volatilized at the injection port and eluted through a capillary column. When the sample moves through the column, due to their affinity for the stationary phase, the various components are separated and can be identified by their retention time. The retention time is defined as time taken for the compound to pass through the column in the gas chromatograph system. Each chemical compound in the sample has a specific retention time measured in minutes which are shown as peaks in a graph and measures abundance on the ordinate against retention time on the abscissa and the peak is correlated to the concentration of the chemical compound. The computer search of the mass spectra which corresponds to the peaks for the sample should yield a statistical match for nicotine at a 12.9min retention

time value and there is a scan mode which looks at all the constituents of a sample, listing whatever chemical components are present.

Compound identification

The molecules of the ethanolic extracts of the drug were identified by comparing their mass spectra and retention indices with those published in the literature and contained in the NIST 2005 MS computer library (Wiley). The active molecules present in salmali are Cinnamic acid-m-derivative (RT value-39.548), Benzimidazole-3-derivative (RT value-39.548), Pyrazole (RT value-39.258), Metolachlor (RT value-38.963), Triamterene (RT Value-38.963) and Nitrazepam (RT Value- 38.963). The results of the TLC analysis is presented in Table 3 indicated the abundance of phytosterol as compared to Alkaloids. The antimicrobial effect on various microorganisms using *Salmali* is indicated in Table 2.

Table 3

Compound	Mobile phase	Ratio	R _f value
Phytosterol	Hexane:Ethyl acetate	5:2	0.5
Alkaloids	Acetone:Ethyl acetate	4:6	0.7

The GC MS analysis indicated the presence of Cinnamic acid, m- derivative (RT value-39.548), Benzimidazole 3-derivative (RT value-39.548), Pyrazole (RT value-39.258), Metolachlor (RT values-38.963), Triamterene (RT Value-38.963) and Nitrazepam (RT Value-38.963) among other minor compounds. The results revealed that the extract is potent antimicrobial against all the microorganisms studied and it has great potential against *Candida albicans*. The antioxidant property of salmali, as revealed in our results, could be attributed to the presence of Cinnamic acid, which is a known antioxidant. The TLC results clearly showed that phytosterols were abundant compared to alkaloids. Regular intake of dietary phytosterol has been reported to reduce the cardiac arrest risk.[21,22,23] The claim that Salmali has cardiogenic and antiangiogenic properties could be attributed to the presence of phytosterols in it.

Cinnamic acid is a key intermediate in shikimate and phenyl propanoid pathway which is a precursor of many aromatic amino acid, alkaloids and indole derivatives. The biological activities of cinnamic acid derivatives are reported be anti TB, Antidiabetic, antioxidant, antimicrobial, as a fragrance material, hepatoprotective, CNS depressant, anticholesteromic, antigungal, fungitoxic, antihyperglycemic, antimalarial, antiviral, anxiolytic, cytotoxic, anti-inflammatory.[24, 25, 26] It is also a U-V ray absorbent.^[27] The derivatives of Cinnamic acid are reported to have strong antioxidant effects.[28, 29]

The p- hydroxy and methoxy groups in cinnamic acid derivatives also showed good insulin releasing activity.[30,31] Due to the presence of Esters, amides and substituted derivatives of cinnamic acid it also shows anti microbial activity.[32] The role of hepato-protective property and CNS depressant activity is due to the presence of Hydroxy cinnamic acid and halogenated cinnamic acid and 3- phenyl propionyl moiety will result in anti malarial activity.[33] The lipid lowering efficacy by the derivatives of cinnamic acid showed anticholesterolemic activity.[34,35,36] The derivatives of cinnamic acid also possess to have anti viral and antifungal property.[37] Morronside cinnamic acid also showed the anti-inflammatory activity of on E- selectin mediated cell-cell adhesion and the derivative of cinnamic acid also shows anti anxiety action.

Benzimidazole-3-derivatives are used as medicines in the name of Oxcarbazepine and Hydantoin or glucolyurea. These medicines are used as anticonvulsant and mood stabilizing medicines, particularly for anxiety, mood disorders and in epilepsy. This drug is also used in hyperthermia, neuroleptic malignancy, and for intoxication.

Metolachlor is medicine named as Pethidine hydrochloride. This a potent analgesic used for moderate to severe pains particularly postoperative and during labor. Another compound that is predominantly available in salmali is Triamterene. This is a standard medicine as a diuretic which is used for cases of hypertension and odema.[38] The presence of Triamterene in Salmali proves its diuretic and hypotensive activity.

Nitrazepam is a standard hyponotic sedative used to relieve insomnia and anxiety. It has motor impairing, amnesic, anticonvulsant and skeletal muscle relaxant properties. [39] To our knowledge there is no report of the presence of Nitrazepam in the literature available for *Salmali* and it seems we are the first to report this. *Salmali*, thus also have positive role in relieving insomnia and anxiety. The results of antioxidant assays for catalase, reducing power assay and POD assay clearly indicated that Salmali does have a potential antioxidant activity. The results of the TLC

analysis is presented in Table 3 indicated the abundance of phytosterol as compared to Alkaloids. Further work on Salmali is in progress to establish its role as claimed by Ayurveda is under way.

CONCLUSION

It is evident from the above discussion that the drug *Salmali* has a strong antimicrobial and antioxidant properties. The present study has clearly shown that there is a great need to establish the efficacy of Ayurvedic drugs in the light of modern scientific knowledge. Further study will enhance our understanding of the basic mechanisms of the functioning of ayurvedic medicines.

From the above discussion certain points could be surmised:

- a. That *Salmali niryasa* contains five important biomolecules with very specific functions as medicines;
 - b. That the claims made by contemporary and alternative medical practitioners of the medical efficacy of Salmali are in tune with the medical implications of these important biomolecules that are present.
 - c. That, it is quite possible that the dosage that are recommended for using Salmali could be well within permissible limit to minimize the side effects of these molecules;
 - d. that, it is also quite possible that there are side effects which are not statistically and experimentally verified;
 - e. that, it is also possible that due to the presence of some other minor components present in the formulation, the drugs might act synergistically, positively/ negatively,
- More elaborate work is in progress to prove the efficacy of salmali at molecular, physiological, pharmacological and toxicological levels.

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