Scientific Validation on Siddha Sashtric Polyherbal Formulation “Raasa Amirthathy Chooranam” for Tuberculosis - A Review

Vimalan M¹, Antony Duraichi R²

¹PG Scholar, Department of Gunapadam, ²Lecturer, Department of Gunapadam, Government Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu, India.

Corresponding Author: Vimalan M

ABSTRACT

Siddha medicines include 32 types of internal medicines and 32 types of external applications. Herbal drugs are given in the forms like choornam (powder), ilagam, Mathirai (tablets), thylam (oil) and mineral preparations in the form of parpam (white calcinated powder), chendooram (red in color) etc. The siddha formulation ‘Raasa amirthathy chooranam’ is a polyherbal formulation consists of 21 herbal ingredients. According to siddha literature, the ingredients in this formulation have therapeutic effects on Uzhzhurukki can be correlated with tuberculosis in modern medical science. Present review focuses on the composition, traditional uses of the Siddha sashtiric polyherbal formulation “Raasa amirthathy chooranam” along with the scientific analysis of its pharmacological action. The current review provides a background for the various pharmacological actions of the ingredients of the formulation. Analyzing various research articles, the ingredients of this formulation possesses Anti tuberculosis, Anti microbial, Hepato protective, Anti inflammatory, Antioxidant and Immuno modulatory activity which will improve the symptoms of illness as well as prevent the complications of disease. This review explains the phyto constituents and pharmacological actions of each ingredients of Raasa amirthathy chooranam that has been mentioned in Vaiththiya Saarasangirakam, 1968, Page No 433 classical siddha literature.

Key Words: Raasa amirthathy chooranam, tuberculosis, polyherbal formulation.

INTRODUCTION

Herbal medicines as the major remedy in Siddha system of medicine have been used in medical practices since antiquity. The practices continue today because of its biomedical benefits as well as place in cultural beliefs in many parts of the world and have made a great contribution towards maintaining human health. Approximately 60% of the population use traditional medicines to treat medical illnesses.

Tuberculosis is a contagious infection that usually attacks the lungs. It can also spread to other parts of the body. It is caused by bacteria (Mycobacterium tuberculosis). It is curable and preventable. It remains the leading cause of death from an infectious disease among adults worldwide with more than 10 million people becoming newly sick from tuberculosis each year. Herbal drugs are gaining worldwide popularity because of lesser side effects, cost effectiveness and easy availability to poor people particularly in developing countries.

In Siddha sashtiric text Vaiththiya Saarasangirakam, Raasa amirthathy chooranam, a polyherbal formulation is mentioned for Tuberculosis. This review focuses the chemical constituents, therapeutic actions of 21 ingredients of
Raasa amirthathy chooranam to get better results in clinical application in managing Tuberculosis by Siddha aspect.

MATERIALS AND METHODS

Type of Study: Literature Review

RESULTS AND OBSERVATIONS

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<tr>
<td>1</td>
<td>Elattaria cardamomum(F)(^1)</td>
<td>Zingiberaceae</td>
<td>Cineole, limonene, terpinolene, myrcene, linalool, alpha terpinyl acetate</td>
<td>Anti tuberculous, Chemo preventive, Immunomodulatory, Anticancer</td>
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<td>2</td>
<td>Cinnamomum verum(B)(^2)</td>
<td>Lauraceae</td>
<td>Eugenol, E-cinnamyl acetate, cinnamaldehyde, carophyllene oxide</td>
<td>Anti tuberculous, Anti microbial, Antioxidant, Anticancer, Anti inflammatory, Anti diabetic</td>
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<td>3</td>
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<td>Fabaceae</td>
<td>Glycyrrhizin, glabrin, liquiritin, semilicoisoflavone, methoxyfisicofinol</td>
<td>Anti tuberculous, Anti inflammatory, Anti ulcer, Antioxidant, Anti cancer, Hepato protective</td>
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<td>4</td>
<td>Cuminum cyminum(F)(^4,5)</td>
<td>Apiaceae</td>
<td>Cuminaldehyde, cuminol, cymene, cuminoside, cimonene, eugenol</td>
<td>Anti tuberculous, Digestive, Antimicrobial, Hepato protective, Anti inflammatory, Bio enhancer activity</td>
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<td>Nigella sativa(S)(^6)</td>
<td>Ranunculaceae</td>
<td>Thymol, thymoquinone, melanthin, campesterol, longistolene</td>
<td>Anti tuberculous, Anti microbial, Anti oxidant, Antimicrobial, Hepato protective, Renal protective, Immunomodulatory</td>
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<td>Trigonella foenum graecum(S)(^15)</td>
<td>Fabaceae</td>
<td>Trigonelline, diosgenin, prolonmin, Scopoletin, coumarin, phytic acid</td>
<td>Anti microbial, Anti oxidant, Anti diabetic, Hepato protective</td>
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<td>7</td>
<td>Hyoscyamus niger(S)(^16)</td>
<td>solanaceae</td>
<td>Hyoscyamine, hyoscine, scopolamine, atropine</td>
<td>Anti microbial, Anti inflammatory, Analgesic, Sedative</td>
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<td>8</td>
<td>Piper cubeba(F)(^7)</td>
<td>Piperaceae</td>
<td>β – caryophyllene, epi – cubebol, cubebol, Y – cadinene</td>
<td>Anti tuberculous, Anti bacterial, Antioxidant, Nephroprotective, Anti inflammatory</td>
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<td>Piper longum(F)(^8)</td>
<td>Piperaceae</td>
<td>Piperine, piperlongumine, sylvatin, sesamin, pipermonaline, piperundecalidine</td>
<td>Anti tuberculous, Carminative, Immunomodulatory, Antioxidant, Anti inflammatory, Hepatoprotective</td>
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<td>10</td>
<td>Embelia ribes(F)</td>
<td>Myrsinaceae</td>
<td>Embelin, embolic acid, embelinol, christembine, cinnamic acid, rapanon</td>
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<td>11</td>
<td>Crocus sativus(P)(^18)</td>
<td>Iridaceae</td>
<td>Crocin, picrocrocin, crocin, safranal</td>
<td>Antisense, Anti inflammatory, Antioxidant, Antimicrobial</td>
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<td>Nardostachys jatamansi(R)(^9)</td>
<td>Valerianaceae</td>
<td>β – eudesmol, elemol, β –sitisterol, algecin, jatamansin</td>
<td>Anti cancer, Antioxidant, Hepatoprotective, Anti bacterial</td>
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<td>Table 1: Continued…</td>
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<tr>
<td>13 Rubia cordifolia(R) Rubiaceae Rubiadin, manjistin, alizarin, garancin, purpurin, xanthopurpurin</td>
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<td>14 Picrorhiza kurrooa(R) Plantaginaceae Picroside I&amp;II, d – laminol, kutkiol, kutki sterol, apocynin</td>
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<td>15 Piper longum(R) Piperaceae Piperine, piperrhominine, sylvatin, sesamin, pipernonaline, piperundecalidine</td>
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<td>16 Myristica fragrans(Nut) Myristicaceae Myristicin, myristic acid, elemicin, safrole, trimyrysthin, myrcene</td>
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<td>17 Syzygium aromaticum (Bud) Myrtaceae Myricetin, eugenol, eugenol acetate, limonin, ferulic aldehyde</td>
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<td>18 Myristica fragrans(Aril) Myristicaceae Myristicin, myristic acid, elemicin, safrole, trimyrysthin, myrcene</td>
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<td>19 Alpinia galanga(Rh) Zingiberaceae Alpinin, galangol, galangin, campheride, galanolactone</td>
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<td>20 Plumbago zeylanica(R) Plumbaginaceae Plumbagic acid, lupeol, uridine, daucosterol, tachioside, norcanelilline</td>
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<tr>
<td>21 Cassia angustifolia(L) Caesalpiniaceae Rutin, scutellarin, quercimeritrin, sennoside A &amp; B</td>
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**PHARMACOLOGICAL ACTIVITIES OF INGREDIENTS RELATED TO TUBERCULOSIS:**

**Elattaria cardamomum:**

**Antituberculosis activity:**

Mature seeds of E. cardamomum and mature rhizomes of Z. officianale and C. longa were air dried and extracted with 99% ethanol using a Soxhlet apparatus for 6-8 hours. The extracts were concentrated to dryness using a rotary evaporator and tested anti-mycobacterial activity by Luciferase reporter phage (LRP) assay against standard strain of M. tuberculosis H37RV at three different concentrations (25, 250 and 500 μg/ml). The results revealed that all the three plants have potential antituberculosis activity.

**Cinnamomum verum:**

**Antituberculosis activity:**

Antituberculosis activity of extracts was tested by agar dilution & Microscopic Observation of Drug Susceptibility Assay (MODS). 50% inhibition (IC50) was calculated by Resazurine Microtitre Assay (REMA). The combined effect of the extracts with INH was evaluated by checkerboard titrations. Minimum Inhibitory Concentration (MIC) of aqueous & methanolic extracts of both plants showed 10 mcg/ml by agar dilution method. Aqueous (CAS) & methanolic (CMS) extracts of Cinnamomum showed MIC value of 0.5 & 2 mcg/ml respectively.
Anti microbial activity\textsuperscript{25}:

Extraction with an aqueous system from the dried stem barks of \textit{C. verum} yielded 2.5\% of the dried plant. Among 10 test strains of bacteria, \textit{C. verum} showed inhibitory effect on the growth of \textit{Klebsiella pneumoniae ATCC 10031}, \textit{Staphylococcus epidermidis ATCC 12228} and \textit{E. coli ATCC 25922} in an agar diffusion test. The Minimal Inhibitory Concentrations (MICs) and the Minimal Bactericidal Concentrations (MBCs) were in the range of 4-16 and 16-32 g L\textsuperscript{-1}, respectively.

\textbf{Glycyrrhiza glabra:}

\textbf{Anti tuberculosis activity\textsuperscript{26}:}

Bioactivity guided isolation of \textit{Glycyrrhiza glabra} (Leguminosae / Fabaceae) roots resulted in the characterization of 18β-glycyrrhetinic acid as a major anti-tubercular agent. Further, GA-1 was semi-synthetically converted into its nine derivatives, which were in-vitro evaluated for their antitubercular potential against \textit{Mycobacterium tuberculosis} H37Rv using BACTEC-460 radiometric susceptibility assay. All the derivatives were active, but the benzylamide (GA-8, MIC 12.5μg/ml) and ethyl oxylate (GA-3, MIC 25.0 μg/ml) derivatives were significantly active against the pathogen. This was further supported by the molecular docking studies, which showed adequate docking (LibDock) scores for GA-3 (120.3) and GA-8 (112.6) with respect to the standard anti-tubercular drug, rifampicin (92.94) on the DNA-directed RNA polymerase subunit beta (rpoB) target site. Finally, the in silico pharmacokinetic and drug-likeness studies showed that GA-3 and GA-8 possesses drug-like properties.

\textbf{Hepato protective activity\textsuperscript{27}:}

\textit{Glycyrrhiza glabra} extract was added to the carp primary hepatocytes before (pre-treatment), after (post-treatment) and both before and after (pre- and post-treatment) the incubation of the hepatocytes with CCl\textsubscript{4}. CCl\textsubscript{4} at 8 mM in the culture medium produced significantly elevated levels of lactate dehydrogenase (LDH), glutamate oxalate transaminase (GOT), glutamate pyruvate transaminase (GPT) and malondialdehyde (MDA) and significantly reduced levels of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). Pre-treatment (5 μg/ml) and pre- and post-treatment (5 and 10 μg/ml) of the hepatocytes with \textit{Glycyrrhiza glabra} extract significantly reduced the elevated levels of LDH, GOT, GPT and MDA and increased the reduced levels of SOD and GSH-Px by CCl\textsubscript{4}; post-treatment of the hepatocytes with \textit{Glycyrrhiza glabra} extract at 5 μg/ml reduced the GPT and GOT levels and increased the GSH-Px level, but had no effect on the other parameters at all the studied concentrations.

\textbf{Cuminum cyminum:}

\textbf{Anti tuberculosis activity\textsuperscript{28}:}

Essential oils were used with cumin, cloves, cinnamon, laurel and anis to determine Minimum Inhibitory Concentration (MIC) against \textit{Mycobacterium tuberculosis} strains. The MICs were determined on \textit{M. tuberculosis} H37Rv sensitive to all five first line antituberculosis drugs (streptomycin, isoniazid, rifampicin, ethambutol and pyrazinamide), two H37-Rv (CH -8 and CH-15) isoniazid-resistant, two H37Rv (CH-07 and CH-09) rifampicin-resistant, two H37Rv (CH-03 and CH-06) streptomycin-resistant, and two H37Rv (CH-09 and CH-10) ethambutol-resistant using the microplate alamar blue assay. The results obtained showed that the cumin and cinnamon essential oils showed a MIC of 12.5 μg/ml against reference strain H37Rv.

\textbf{Hepato protective activity\textsuperscript{29}:}

Administration of cisplatin raised the level of LFT’s enzymes and also reduced the level of antioxidant enzymes in the liver of the mice. Administration of \textit{Cuminum cyminum}, \textit{Phyllanthusemblica} extract and silymarin remarkably showed the hepatoprotective effect in the albino mice. Administration of \textit{C. cyminum}, \textit{P. emblica}
and silymarin decreased the level of ALT, AST, and ALP along with increasing the level of Total protein content. It also increased the level of antioxidant enzymes in the liver of mice showing its hepatoprotective activity. We found C. cymimum has a better hepatoprotective effect than P. emblica.

**Anti microbial activity** 30:
Three groups of microorganisms [aerobic bacterial mixture, anaerobic bacterial mixture and Enterococcus faecalis (E. faecalis)] were isolated from the teeth with persistent apical periodontitis. Zone of inhibition (ZOI), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum biofilm inhibitory concentration (MBIC) and time-kill tests were performed to assess the antimicrobial efficacy of the medicaments. Further, a cytocompatibility analysis of the medicaments was performed on L929 fibroblasts. Co-trimoxazole showed the greatest ZOI followed by cumin and CHX. The smallest MIC and MBC belonged to co-trimoxazole followed by cumin and CHX for all groups of bacteria except for E. faecalis for which the MBC of cumin was smaller than co-trimoxazole. The results of time-kill assay revealed that all medicaments totally inhibited the bacterial growth in all groups after 24 h. CHX was the most cytotoxic solution while there were no significant differences between the cytocompatibility of different concentrations of cumin essential oil and co-trimoxazole.

**Nigella sativa:**

**Anti tuberculosis activity** 31:
In Middlebrook broth, fluorescence test for tuberculosis was negative with thymoquinone 20, 40 and 80 μg/ml and streptomycin 1.25 μg/ml up to day 14th. With controls, thymoquinone 2.5 and 5 μg/ml and streptomycin 1.25 μg/ml, however, with controls, thymoquinone 2.5 and 5 μg/ml abundant and with 10 μg/ml few colonies were observed.

**Anti microbial activity** 32:
Filter paper discs impregnated with the diethyl ether extract of *Nigella sativa* seeds (25–400 μg extract/disc) caused concentration dependent inhibition of Gram-positive bacteria represented by *Staphylococcus aureus*. Gram-negative bacteria represented by *Pseudomonas aeruginosa* and *Escherichia coli* (but not *Salmonella typhimurium*) and a pathogenic yeast *Candida albicans*. The extract showed antibacterial synergism with streptomycin and gentamicin and showed additive antibacterial action with spectinomycin, erythromycin, tobramycin, doxycycline, chloramphenicol, nalidixic acid, ampicillin, lincomycin and sulphasemethoxazole-trimethoprim combination. The extract successfully eradicated a non-fatal subcutaneous staphylococcal infection in mice when injected at the site of infection.

**Hepato protective activity** 33:
The in vitro and in vivo findings of this study demonstrated that the NSSE has protective effects against APAP-induced hepatotoxicity and metabolic disturbances by improving antioxidant activities and suppressing both lipid peroxidation and ROS generation.

**Trigonella foenum graecum:**

**Anti microbial activity** 34:
The inhibitory effect of fenugreek oil was tested against four microorganisms: three bacteria: *Escherichia Coli*, *Staphylococcus aureus*, and *Salmonella typhimurium* and one Mould: *Aspergillus niger*. The results indicated that the oil has a Potent antimicrobial activity against all tested microorganisms. The Highest antimicrobial activity among the bacteria was detected Against E. coli, where the inhibition zone diameter was 20 mm.
However, the highest antimicrobial activity against all tested Organisms was found against Aspergillus niger where a complete Inhibition (100%) was recorded.

Hepato protective activity:35

Hepatic injury in rats was induced separately by administration of equal mixture of CCI4 and olive oil (50% v/v, 1.25 ml/kg, ip). Liver damaged was monitored by raised biochemical marker enzymes (SGOT, SGPT, and alkaline phosphatase). CCI4 was administered twice a week, on every first and fourth day of all 14 days. The extract at the dose of 250 mg/kg b. wt. was evaluated by inducing hepatotoxicity with CCI4 and using silymarin (100 mg/kg) as the reference standard. Biochemical parameters like, SGOT, SGPT and serum bilirubin level were analysed. A section of liver was subjected to histopathological studies. Based on the above studies, it is reported that the methanol extract of TFG possess significant hepato-protection against CCI4 induced hepatotoxicity in albino rats.

Piper cubeba:
Antibacterial activity:36

The evaluation of the antibacterial activity has been done by broth microdilution technique for determination of the minimum inhibitory concentration and the minimum bactericidal concentration against Porphyromonas gingivalis, Prevotella nigrescens, Actinomyces naeslundii, Bacteroides fragilis and Fusobacterium nucleatum. It was possible to make an analysis regarding the relationship between structure and antimicrobial activity of derivatives against microorganisms that cause endodontic infections. The most promising were minimum inhibitory concentration =50 µg/ml against P. gingivalis by (2) and (3), and minimum inhibitory concentration =100 µg/ml against B. fragilis by (6). Cytotoxicity assays demonstrated that (1) and its derivatives do not display toxicity.

Anti tuberculosis activity:37

A novel piperine dimer, named chabamide, was isolated from stems of Piper cubeba Hunter and its structure was elucidated on the basis of spectroscopic evidence. Chabamide showed antimalarial activity with an IC 50 value of 2.7 µg/ml and antituberculosis activity with the minimum inhibitory concentration (MIC) of 12.5 µg/ml.

Piper longum:
Hepato protective activity:38

On Administration of the aqueous extract of fruits of Piper longum along with the anti TB drugs, the levels of lipid peroxides in Liver homogenate as well as serum are significantly decreased (p<0.001) compared to the group treated with antitubercular drugs. Similarly on administration of piperine along with anti TB drugs, lipid peroxides in serum and in liver homogenate are significantly reduced (p<0.001) compared to the group treated with antitubercular drugs. The levels of reduced Glutathione are also significantly reduced (p<0.001) in the Group treated with anti tubercular drugs compared to normal Control. Administration of extract of fruits of Piper longum and piperine along with the anti TB drugs significantly increased the levels of reduced glutathione (p<0.001) Histopathological studies indicate no significant change between the control and the group administered with Antitubercular drugs. On extending the study of Hepatoprotective effect to infected animals, it was again observed that there was a significant increase (p<0.001) in lipid peroxides in liver homogenate of animals on Administration of anti TB drugs. The levels were lowered significantly (p<0.001) on administration of extract of fruits of Piper longum with anti TB drugs compared to the group treated with antitubercular drugs. There was a significant decrease (p<0.05) in reduced glutathione levels in the group treated with anti TB drugs. On administration of extract of fruits of Piper longum with anti TB drugs a significant increase (p<0.001) in the levels of reduced
glutathione is observed compared to the group treated with antitubercular drugs.

**Anti tuberculosis activity**

A bioassay guided fractionation of Pippali (Piper longum L.) was performed in five different organic solvents and their activities were monitored against different pathogenic bacteria including MDR Mycobacterium. Different fractions were screened for the bioactivity against Mycobacterium, and the structure of bioactive compound was characterized with H 1 and C 13 NMR. An ethyl acetate fraction of Pippali extract was found active against M. smegmatis (3000 µg ml⁻¹) and M. tuberculosis (39 µg ml⁻¹). It also shows very significant activity against other bacterial strains like E.coli (152 µg ml⁻¹), Staphylococcus aureus (14 µg ml⁻¹), Salmonella typhi (180 µg ml⁻¹), Enterococcus faecalis (15 µg ml⁻¹), and Pseudomonas aeruginosa (52 µg ml⁻¹). This fraction of ethyl acetate was then purified and characterized as piperine [5-(1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one], a well known alkaloid from this plant. Bioactivity guided fractionation concludes that Piperine is the only active ingredients in various fractions of fruit extract evaluated for antibacterial activity.

**Embelia ribes:**

**Antimicrobial activity**

The antimicrobial activity against two micro-organisms were tested using disc diffusion method and cytotoxicity of GNPs and SNPs was determined against MCF-7 cell lines at different concentrations by MTT assay. Both the GNPs and SNPs prepared from E. ribes comparatively showed promising results thereby proving their clinical importance.

**Crocus sativus:**

**Antimicrobial activity**

The antimicrobial activity was determined by the evaluation of the minimum inhibitory concentration using the agar well plate procedure. The most effective extract was SPE with a minimum inhibitory concentration varying between 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.63 µg/mL.

**Nardostachys jatamansi:**

**Hepatoprotective activity**

A 50% ethanolic extract of the rhizomes of N. jatamansi is shown to possess hepatoprotective activity. Pretreatment of rats with the extract (800 mg/kg body wt, orally) for three consecutive days significantly ameliorated the liver damage in rats exposed to the hepatotoxic compound thioacetamide. Elevated levels of serum transaminases (aminotransferases) and alkaline phosphatase, observed in thioacetamide alone treated group of animals, were significantly lowered in N. jatamansi pretreated rats. Pretreatment of the animals with the extract also resulted in an increase in survival in rats intoxicated with LD90 dose of the hepatotoxic drug.

**Rubia cordifolia:**

**Anti-mycobacterial activity**

Anti-mycobacterial activity of Rubia cordifolia leaves were tested on DCM and ethanolic extracts against H37Rv strain of Mycobacterium tuberculosis using the green fluorescence protein microplate assay (GFPMA). The ethanolic crude extract (K2F-3) was less active than the DCM (K2F-1) crude extract, with MIC90 of 57.3 and 44.5 µg/ml respectively as shown in Table 1 and Table 2 respectively.
Fractionation of the crude extracts by preparative chromatography, eluting with 5% methanol-DCM afforded four sub-fractions from each crude. The sub-fractions from the ethanol crude demonstrated better activity compared to those obtained from the DCM crude.

**Hepato protective activity**

Rubiadin at a dose of 50, 100 and 200 mg/kg was administered orally once daily for 14 days. The substantially elevated serum enzymatic activities of serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) and γ-glutamyltransferase (γ-GT) due to carbon tetrachloride treatment were dose dependently restored towards normalization. Meanwhile, the decreased activities of glutathione S-transferase and glutathione reductase were also restored towards normalization. In addition, rubiadin also significantly prevented the elevation of hepatic melondialdehyde formation and depletion of reduced glutathione content in the liver of CCl4 intoxicated rats in a dose dependent manner. Silymarin used as standard reference also exhibited significant hepatoprotective activity on post treatment against carbon tetrachloride induced hepatotoxicity in rats. The biochemical observations were supplemented with histopathological examination of rat liver sections. The results of this study strongly indicate that rubiadin has a potent hepatoprotective action against carbon tetrachloride induced hepatic damage in rats.

**Anti microbial activity**

55 plant methanolic extracts were investigated for antimicrobial activity against, thirteen phytopathogens of Gossypium using agar ditch diffusion method. Results from the in vitro antimicrobial assays indicated that six plant extracts exhibited antimicrobial activity, in which highest activity was observed from the root extracts of R. cordifolia and G. glabra. Qualitative phytochemical tests, Column chromatography and of these two active root extracts demonstrated the presence of phyto compounds VIZ, anthraquinones and flavonoids as major active constituents respectively.

**Picrorhiza kurroa:**

**Hepato protective activity**

P. kurroa by itself had no cytotoxic effects on the liver slices at a wide range of concentrations (0.5-0.1%; data not shown). Percent release of LDH from slices treated with the highest concentration (20 mg/ml) of P. kurroa extract alone (8.5%± 0.14) was found to be similar to that in the case of control Untreated slices (10.2%±2.6) [Table 3]. In all the experiments, Three concentrations of P. kurroa, viz., 20 mg/ml, 10 mg/ml and 0.5 mg/ml were used, while the time course for the release of cytotoxicity marker enzyme was followed only for the highest concentration of P. kurroa (20 mg/ml). P. kurroa, when added along with ethanol, inhibited the release of all three marker enzymes in a concentration-dependent manner. Ascorbic acid (10 mM) was used as a standard antioxidant and protected the liver slices from the cytotoxic effect of ethanol.

**Piper longum (root):**

**Anti bacterial activity**

Antibacterial activity of piperlongumine was evaluated against 18 clinically isolated strains, including identified strains belongs to Klebsiella pneumoniae, Pseudomonas aeruginosa and Staphylococcus aureus, using the agar-well diffusion method. All the clinical strains showed concentration dependent susceptibility towards the constituent piperlongumine (25, 50, 100μg/100μL). It exhibited significant zone of inhibition against both the clinical and ATCC and MTCC strains. The antibacterial activity was more pronounced against Klebsiella pneumonia (24.00±0.12 mm) and Pseudomonas aeruginosa (20.00±0.12 mm)
while it was moderate on Staphylococcus aureus (16.47±0.18 mm).

**Myristica fragrans: Anti microbial activity**

Acetone extract has shown the strongest antibacterial and antifungal activity with Staphylococcus aureus (13.8 ± 0.42 mm) and Aspergillus niger (14.4 ± 0.37 mm), respectively. GC–MS analysis of acetone extract has revealed the presence of 32 compounds of extract representing 99.49%. Sabinene (28.61%) has shown the highest occurrence in the extract. B-Pinene (10.26), α-pinene (9.72), myristicin (4.30%), isoeugenol (2.72%), p-cymene (1.81%), carvacrol (1.54%), eugenol (0.89%) and β-caryophellene (0.82%) were reported as possible contributor for antioxidant and antimicrobial activity of nutmeg.

**Hepatoprotective activity**

Myristicin, one of the major essential oils of nutmeg, was found to possess extraordinarily potent hepatoprotective activity. Myristicin markedly suppressed LPS/D-GalN-induced enhancement of serum TNF-α concentrations and hepatic DNA fragmentation in mice. These findings suggest that the hepatoprotective activity of myristicin might be, at least in part, due to the inhibition of TNF-α release from macrophages.

**Syzygium aromaticum: Anti tuberculosis activity**

Methanolic and acetone extract of Syzygium aromaticum exhibited very good anti-TB activity having MIC of 0.8 µg/ml and 12.5 µg/ml respectively.

**Hepato protective activity**

30 Healthy Rabbits weighing 2kg on average were divided into 3 equal groups. Group A was taken as control group having no intervention while group B was given paracetamol 500mg BD for 10 days followed by SyzygiumAromaticum powder 100mg BD for next 10 days. Group C was given paracetamol 500mg BD and Syzygium Aromaticum powder 100mg BD for 20 days. Blood samples were taken from ear lobes through 24 gauge canula for liver enzymes at days 1, 10 and 20 and analyzed in ISRA Laboratory. Mean and standard deviation were calculated and p-value <0.05 was taken as significant. The data was entered and analyzed by using SPSS version 16. There was no rise in liver enzymes in group A at any stage of the study. Liver enzymes ALT, AST, GGT, ALP and LDH markedly increased in group B in initial 10 days but declined in next 10 days. There was no significant rise in liver enzymes in group C at any level of the study.

**Myristica fragrans (aril part): Anti microbial activity**

The zone of inhibition in clove ethanolic extract against all the food associated bacteria was in the range of 25mm to 32mm and in molds the percent mycelial growth inhibition ranged from 70% to 100%.The clove ethanolic extract exhibited the maximum zone of inhibition against E. coli. The MIC values of clove ethanolic extract for different bacterial isolates ranged from 5.0 mg/ml to 20mg/ml and 10 mg/ml to 20mg/ml against molds.

**Alpinia galanga: Anti tuberculosis activity**

1’ acetoxychavicol acetate, the reference standard used, was present in all the three extracts. The acetone and ethanolic extracts were active in axenic (aerobic and anaerobic) and intracellular assays. The aqueous extract did not demonstrate activity under the defined assay parameters.
Plumbago zeylanica: 
Anti tuberculosis activity\textsuperscript{56}: 

Plumbagin analogs, obtained from Plumbago zeylanica (Family: Plumbaginaceae), have been synthesized. Out of the various synthesized analogs, the antitubercular activity of compound a and b was evaluated using standard H 37 Rv and S, H, R, and E sensitive M tuberculosis strains using LRF assay method. Compound a showed strong activity against both standard H 37 Rv and S, H, R and E sensitive M. tuberculosis strains as compared to standard Rifampicin. The other compounds are proved to be more active against standard H 37 Rv and S, H, R and E sensitive M. tuberculosis strain as compared to Rifampicin.

Hepato protective activity\textsuperscript{57}: 

Hepatotoxicity was induced in male Wistar rats by intra peritoneal injection of CCl\textsubscript{4} (0.1 ml/kg/day for 10 days). Extracts of P. zeylanica rhizome were administered to the experimental rats (25 mg/kg/day, po for 14 days). The hepatoprotective effect of these extracts was evaluated by the assay of liver function biochemical parameters (Protein, Cholesterol, Bilirubin, Alanine amino transaminase, and Aspartate amino transaminase activities). In rhizome extract- treated animals, the toxic effect of CCl\textsubscript{4} was controlled significantly by restoration of the levels of Protein, Cholesterol, Bilirubin Alanine amino transaminase and Aspartate amino transaminase as compared to the control cohort, which evidenced the hepatoprotective activity. Rhizome extract of P. zeylanica possesses significant hepatoprotective activity.

Cassia angustifolia: 
Anti microbial activity\textsuperscript{58}: 

Methanolic extraction of plant showed notable antifungal activities against Aspergillusniger, Aspergillus terreus, Aspergillus flavus, and Aspergillus fumigatus C.Angustifolia was very highly active against A. terreus(6.01±0.27). Aspergillus was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug amphotericin B and Fluconazole to some extent.

CONCLUSION 

This review especially exposes the ingredients of \textit{Raasa Amirthathy Chooranam} have Anti tuberculosis, Anti microbial, Hepato protective, Anti inflammatory, Antioxidant and Immuno-modulatory activity. These pharmacological activities and Chemical constituents contribute major role in treatment of tuberculosis. Furthermore this formulation should be subjected to preclinical evaluations to understand the safety and better effectiveness of herbal medicine before prescribing to the patients.

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