



PRELIMINARY PHYTOCHEMICAL ANALYSIS OF A POLYHERBAL SIDDHA FORMULATION-ROJAMOGGU LEHYAM

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ABSTRACT

Rojamoggu Lehyam (RML) is a polyherbal siddha medicine. RML is used in treating gastritis and peptic ulcer for more than 3 decades. The present study is made to investigate the phytochemical bio active components present in the aqueous extract. The extract is prepared by the standard procedure. This study is the initial level of understanding the types of bio active compounds present in RML and further work is to be carried out to find out the more medicinal efficacy by other standard methods. The results of this study shows that the carbohydrates, cardiac glycosides, saponins, phenols, tannins, flavonoids, diterpenes, gum mucilage, fixed oils & fats were present and absence of alkaloids, phytosterols, proteins & amino acids and quinines in the RML aqueous extract.

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INTRODUCTION

Ancient system of medicine such as Siddha is a time-tested system of medicine which has been in clinical use for centuries in India. Being a time-tested system, it has an edge over other existing systems of health management. When two or more herbs are used in formulations, they are known as polyherbal formulations. Siddha medicine has roots in medicinal herbs and they have been practiced for centuries. Herbal medicine is making dramatic comeback and increasing number of patients are visiting Indian system of medicine clinics. Side effects of synthetic medicine are alarming and recent time has seen risk of herbal and herbal-synthetic drug interactions⁽¹⁾.

Herbal formulation might overcome resistance of antibiotics. Poly herbal formulation possess chemical substances generally termed as bioactive compounds including alkaloid, carbohydrates, flavonoids, phenols, tannins, saponins, steroids, protein, aminoacids, etc., which give definite physiological activity on human bodies⁽²⁾.

Rojamoggu lehyam (RML) is manufactured by Lakshmi seva sangham, a GMP and ISO 9001:2000 certified Siddha Ayurveda pharmacy and it is widely used in treating Gastritis & Peptic ulcer since 1982. This study is to identify the bioactive components present in the RML and its uses in treating the diseases as said above. All though no side effects are reported it is not advisable for diabetics since it contains sugar.

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Bioactive compounds such as alkaloids, flavonoids, terpenoids, sterols, carbohydrates, saponins and phenolic compounds were detected to be present in the roots of *Coriandrum sativum* plant⁽³⁾. *Rosa centifolia* Linn is one such plant that is commonly found throughout India. It is extensively used as traditional medicine in Uttar Pradesh and Bihar. A decoction of flowers of rose is prescribed for inflammation of the mouth and pharynx, and ulcers of the intestine. Powder of rose buttons and seeds is used as astringent in haemorrhage and diarrhoea. Studies clearly explain about the anti ulcer activity of *Rosa centifolia*. The ethanol extract of *Rosa centifolia* produced significant anti-tussive activity. The essential oil obtained from *Rosa centifolia* is reported gastro intestinal relaxant activity; therefore the bronchodilatory effect is responsible for its anti-tussive property and might be due to its possible tachykinin inhibitory substance mediating anti-tussive effect⁽⁴⁾.

The data also suggest that the pericarp of the fruit of *Terminalia chebula* is highly nutritious and could be a source of dietary supplement being rich in carbohydrates and amino acids⁽⁵⁾. Research has been done with amla (*Emblica officinalis*) evaluating its role as an antioxidant in ulcer prevention for people with diabetes for mental and memory effects and its anti-inflammatory benefits. Amla extract supplements are helpful in those undergoing radiation therapies or would it also protect the cancer cells that the radiation is trying to destroy⁽⁶⁾. The anti-inflammatory activity of methanol extract of leaves was studied in carrageenan induced paw oedema as well as immunologically induced paw oedema and also its anti-ulcer activity was studied in albino rats. The activity was found to be significant

when compared to the standard drugs. The combination of methanol extract and phenylbutazone (Non-steroidal anti-inflammatory drug, NSAID) was found to have the most potent anti-inflammatory activity experimentally with least toxic (no ulcerogenic) activity. Thus the combination of herbal product i.e. methanol extract of *Bambusa bambos* with modern medicine (NSAID) will produce the best anti-inflammatory drug and will be useful for long term treatment of chronic inflammatory conditions. The anti-inflammatory activity can be attributed to the presence of α -Amyrin and phenolic compounds⁽⁷⁾. *Santalum album* is one of the traditionally used anti-ulcer plants in Unani medicine and a component of certain polyherbal preparations for prevention and healing of gastric ulcers Preliminary phytochemical screening reveals the presence of secondary metabolites like alkaloids, anthraquinone glycosides, saponins, tannins and terpenes⁽⁸⁾.

MATERIALS AND METHODS

RML is collected from a nearby Indian medicine shop at Dindigul. Source of RM lehyam is Lakshmi seva sangham, a GMP and ISO 9001:2000 certified Siddha Ayurveda pharmacy, Gandhigram.

Ingredients of RML are *Rosa centifolia*, *Coriandrum sativum*, *Terminalia chebula*, *Emblica officinalis*, *Santalum album*, *Bambusa arundinacea*, *Saccharum officinarum* and Honey.



Fig 1 Images of Ingredients of Rojamoggu lehyam

Determination of Extractable method

Determination of water-soluble extraction

5 gm of the RML is macerated with 100 ml of distilled water in a closed flask for 24 hrs, shaken frequently for the first six hours in shaker and allowed to stand for the next 18 hrs. The macerate should be filtered rapidly, taking precautions against loss of solvent.

25 ml of this filtrate is evaporated to dryness in a flat bottom shallow dish and dried at 105° Celsius and collected⁽⁹⁾. The extract thus obtained is used for the phytochemical analysis.

Preliminary Phytochemical Test

The preliminary phytochemical screening test was carried out for each extracts of RML as per the standard procedure mentioned^(3, 10).

Detection of alkaloids

Extracts were dissolved individually in diluted hydrochloric acid and filtered.

Mayer's test

2 ml of extract was treated with few drops of Mayer's reagent, formation of yellow coloured precipitate indicates the presence of alkaloids

Wagner's test

2 ml of filtrate was treated with Wagner's reagent. Formation of brown/reddish precipitate indicates the presence of alkaloids.

Detection of Carbohydrate

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for presence of carbohydrates.

Molisch's test

2 ml of filtrate was treated with few drops of alcoholic Alpha naphthol solution in a test tube. Formation of the violet ring at the junction indicates presence of carbohydrates.

Benedict's test

Filtrate was treated with Benedict's reagent and heated gently.

Orange red precipitate indicates the presence of reducing sugars

Fehling's test

Filtrates was treated with fehling solution A and B and the mixture is shaken well and placed in boiling water for a while a brick red precipitate indicates the presence of reducing sugar.

Detection of Glycosides

Liebermann's test

2ml of extract was treated with 2ml chloroform and 2ml of acetic acid, Violet colour change into blue and green indicates presence of Glycosides.

Detection of Saponins

Froth test

Extracts was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of Saponins.

Foam test

0.5gram extract was shaken with 2 ml of water. If foam produced persists for 10 minutes, it indicates the presence of Saponins.

Detection of phytosterols

Salkowski's test

Extracts was treated with chloroform and filtered; the filtrates were treated with few drops of concentrated sulphuric acid, shaken and allowed to stand for few minutes. Golden yellow colour indicates the presence of triterpenes.

Detection of phenols

Ferric chloride test

2 ml of extracts was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Detection of tannins

Gelatin test

To the extracts, 1% of gelatin solution containing sodium chloride was added, formation of white precipitate indicates the presence of tannins.

Detection of flavonoids

Alkaline reagent test

Extract was treated with few drops of 10% sodium hydroxide, formation of intense yellow colour then on addition of diluted hydrochloric acid it becomes colourless, it indicates the presents of flavonoids.

Lead acetate test

Extract was treated with few drops of lead acetate solution, yellow colour precipitate indicates presence of flavonoids.

Detection of protein and aminoacids

Xanthoproteic test

Extracts were treated with 1ml of the amino acid solution taken in a test tube, add few drops of nitric acid and mix the contents. Boil the contents over a Bunsen flame, using a test tube holder, for few minutes. Cool the test tube under running tap water and add few drops of alkali. White colour precipitate indicates the presence of aminoacids.

Ninhydrin Test

Extracts were treated with 1ml of amino acid solution taken in a test tube, add few drops of ninhydrin reagent and mix the contents. Place the test tube in a boiling water bath for 5 minutes and cool to room temperature. Blue colour indicates the presence of aminoacids.

Detection of Diterpenes

Copper Acetate test

Extracts were dissolved in water and treated with 3-4 drops of copper Acetate solution, formation of emerald green colour indicates the presence of diterpenes.

Detection of gum and mucilage

The extract was dissolved in 10 ml of distilled water and to this 2ml of absolute alcohol with the constant stirring white cloudy precipitate indicates the presence of gum and mucilage.

Detection of quinines

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

Detection of Fixed oils and Fats

Oil Stain test

The extracts were pressed between two filter papers. Oil stains on the papers indicated the presence of fixed oils.

Tab 1 Results of preliminary phytochemical analysis of Aqueous extracts of RML

| S.No | Photochemicals | Test Name | Result | Aqueous extract |
|------|----------------------------------|---------------------------|--|-----------------|
| 1 | Alkaloids | Mayer's test | yellow precipitate | - |
| | | Wagner's test | Brown/Reddish precipitate | - |
| 2 | Carbohydrates Reducing sugars | Molisch's test | Violet Ring at the junction | + |
| | | Benedict's test | Orange-Red precipitate | + |
| | | Fehling's Test | Red Precipitate | + |
| 3 | Glycosides Cardiac Glycosides | Libermann burchard's test | Violet colour turns to Blue to Green | - |
| | | Keller – Killiani Test | Brown Ring in the interface | + |
| 4 | Saponins | Froth test | Formation of 1 cm layer foam | + |
| | | Foam test | Foam produced persists for 10 mins | + |
| 5 | Phytosterols | Salkowski's test | Golden yellow colour | - |
| 6 | Phenols | Ferric chloride test | Bluish black colour | + |
| 7 | Tannins | Gelatin test | White precipitate | + |
| 8 | Flavonoids | Alkaline reagent test | Intense yellow colour on addition of dil.acid becomes colourless | + |
| | | Lead acetate test | Yellow precipitate | + |
| 9 | Proteins & Amino acids | Xanthoproteic test | White precipitate | - |
| | | Ninhydrin Test | Blue colour | - |
| | | Copper acetate test | Emerald green colour | + |
| 10 | Diterpenes | | Emerald green colour | + |
| 11 | Gum mucilage | | White cloudy precipitate | + |
| 12 | Quinones | | Blue/red precipitate | - |
| | | Spot Test | Indication of Oil stains | + |
| 13 | Fixed Oils and Fats | | Formation of Soap or partial neutralization of Alkali | + |
| | | Saponification test | | |

+ = Present - = Absent

Saponification test

To the extracts, few drops of 0.5N alcoholic potassium hydroxide and a drop of phenolphthalein were added and heated on a water bath for 1-2 hours. Formation of soap and/or partial neutralization of alkali indicated the presence of fixed oils and fats.

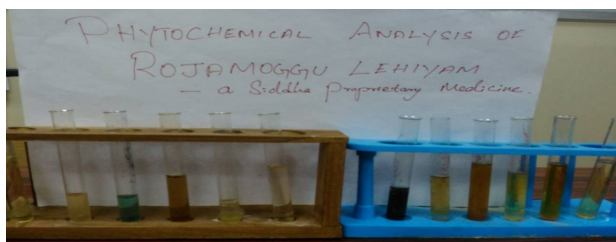


Fig 2 Phytochemical Analysis of Aqueous extract of RML

RESULT

The preliminary phytochemical study of extract of RML - a polyherbal siddha medicine by using aqueous solvent was done using the standard procedures. The results were presented in tables. The present study reveals that the bioactive compounds like carbohydrates, cardiac glycosides, saponins, phenols, tannins, flavonoids, diterpenes, gum mucilage, fixed oils & fats were present and absence of alkaloids, phytosterols, proteins & amino acids and quinines in the RML aqueous extract.

DISCUSSION AND CONCLUSION

RML is a polyherbal formulation which has many therapeutic effects. Since it is administered internally along with water, aqueous extract has been prepared by standard procedure to find the preliminary phytochemical analysis of bioactive compounds present. Flavonoids, Tannins, Phenol present in the RM lehyam extract are a major group of phytochemicals which act as primary anti-oxidants⁽²⁾. Presence of saponins stimulates the immune system and also lowers the blood cholesterol level⁽¹¹⁾. Naturally occurring diterpenes exert several biological activities such as anti-inflammatory action, antimicrobial and antispasmodic activities⁽¹²⁾. Presence of fat helps in promoting healthy cell function. Presence of Gum mucilage prevents the too rapid emptying of the stomach contents into the small intestine thereby improving absorption of certain nutrients in the small intestine⁽¹³⁾. Carbohydrates act as antioxidants and prevent the body from the harmful effects of free radicals. They also protect the body against many bacterial and viral diseases.

Based on these bioactive compounds present in this RML it is scientifically proven that this RML is effective for Gastro intestinal tract disorders. Further clinical studies were continued in this polyherbal siddha medicine for biological activities to find out amazing results in treating Peptic ulcers and thereby helping the global mankind.

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References

1. Rajasree, P.H *et al.* 2012. Formulation and evaluation of antiseptic polyherbal ointment. *Int. J. of Pharmacy & Life Sciences.*, 3(10): 2021-2031.
2. Uma kullappa shanmugam *et al.* 2017. Preliminary phytochemical analysis of siddha formulation – Parangipattai kudineer. *Eur. J. of Biomedical and pharmaceutical sciences.*, 4(1): 526-530.
3. Sasikumar, R *et al.* 2014. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Coriandrum sativum* L. Roots (Coriander). *J. of Pharmacognosy and Phytochemistry.*, 2(6): 74-78.
4. Jena Jitendra *et al.* 2012. *Rosa centifolia*: Plant review. *Int. J. of research in pharmacy and chemistry.*, 2(3): 794-796.
5. Roopalatha, U. C. and Vijay Mala (Grover) Nair. 2013. The Phytochemical Screening of the Pericarp of Fruits of *Terminalia Chebula* Retz., *Int. J. Pharm Bio Sci.*, 4(3): 550 -559.
6. Virendra Yadav *et al.* 2014. Amla (*Embllica officinalis*)- Medicinal food and Pharmacological activity. *Int. J. of Pharmaceutical And Chemical Science.*, 3(3): 616-619.
7. Kaikini Aakruti, A *et al.* 2013. Overview of Indian Medicinal tree: *Bambusa Bambos*. *Int. Research J. Of Pharmacy.*, 4(8): 52-55.
8. Nazeer ahmed *et al.* 2013. Anti-ulcer Activity of Sandalwood (*Santalum album* L.) Stem Hydroalcoholic Extract in Three Gastric-Ulceration Models of Wistar Rats. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas.*, 12(1): 81-91.
9. Agarwal, S.S. and Paridhavi, M. 2012. Herbal Drug Technology. Standardisation of herbal drugs Second edition., 10(2): 651.
10. Syed, M. Salman *et al.* 2015. Preliminary phytochemical essential element analysis and antimicrobial activities of ethanolic extract of *Lotus Corniculatus*. *Int. J. of Biosciences.*, 7(2): 106-115.
11. Jyothi, K.S. and Seshagiri, M. 2012. In-Vitro Activity of Saponins of *Bauhinia Purpurea*, *Madhuca Longifolia*, *Celastrus Paniculatus* and *Semecarpus Anacardium* on Selected Oral Pathogens. *J. dentistry, Tehran university of medical sciences.*, 9(4): 216-223.
12. Sergio, R. Ambrosio *et al.* 2006. Kaurane and pimarane-type diterpenes from the *Viguiera* species inhibit vascular smooth muscle contractility. *Life sciences.*, 79(10): 925-933.
13. Azhar Jabeen *et al.* 2014. Flaxseed/*Tukhm-e-katan Linum usitatissimum* Linn.: A Review. *J. Pharmaceutical and Scientific Innovation.*, 3(5): 401-409.
