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Preliminary Phytochemical Screening of Poly Herbal Formulation *Nalla marunthu.*

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ABSTRACT

Infectious diseases are a significant cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths in tropical countries and as much as 20% of deaths in the Americas. Despite the significant progress made in microbiology and the control of microorganisms, sporadic incidents of epidemics due to drug resistant microorganisms and hitherto unknown disease-causing microbes pose an enormous threat to public health. These negative health trends call for a global initiative for the development of new strategies for the prevention and treatment of infectious disease. The reasons for this renaissance include a reduction in the new antibacterial drugs in the pharmaceutical pipeline, an increase in antimicrobial resistance, and the need of treatments for new emerging pathogens. Literally thousands of plant species have been tested against hundreds of bacterial strains in vitro and many medicinal plants are active against a wide range of micro organisms. Therefore, it is of interest to investigate the various phytochemical constituents of aqueous methanolic, aqueous chloroform and aqueous ethyl acetate extracts of *Nalla marunthu* were screened. Our results indicate that the presence of phytochemical constituents such as alkaloids, flavanoids, steroids, tannins and cardiac glycosides.

Keywords: Infectious diseases , Epidemics, Antimicrobial agents, Emerging pathogens, *Nalla marunthu*.

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INTRODUCTION

The International Agency for Research on Cancer estimates of the incidence of mortality and prevalence from major types of cancer, at national level, for 184 countries of the world revealed that there were 14.1 million new cancer cases, 8.2 million cancer deaths, and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide (1). By 2030, it is projected that there will be 26 million new cancer cases and 17 million cancer deaths per year. Today, despite considerable efforts, cancer still remains an aggressive killer worldwide. Moreover, during the last decade, novel synthetic chemotherapeutic agents currently in use clinically have not succeeded in fulfilling expectations despite the considerable cost of their development (2).

Therefore, there is a constant demand to develop new, effective, and affordable anticancer drugs (3). From the dawn of ancient medicine, chemical compounds derived from plants have been used to treat human diseases. Natural products have received increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents. In parallel, there is increasing evidence for the potential of plant-derived compounds as inhibitors of various stages of tumorigenesis and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy (4). Therefore, it is of interest to investigate the various phytochemical constituents of aqueous methanol, and chloroform extracts of *Nalla marunthu* were screened.

MATERIALS AND METHODS

Collection of samples

The poly herbal formulation (*Nalla marunthu*) is used for the experiment. The herbal formulation was prepared by the available literature.

Preparation of extracts

500 grams of *Nalla marunthu* was packed in three separate round bottom flask for sample extraction using solvents namely Aqueous, Chloroform and Methanol. The extraction was conducted by 250 ml of the each solvent mixture for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and keep it in water bath (at 50°C). Now the extracted experimental solutions were stored in refrigerator.

Phytochemicals analysis

The extracts were prepared and analyzed for the presence of alkaloids, saponins, tannins, steroids, flavonoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature (5-12).

Test for alkaloids

The extract of the crude dry leaf powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrochloric acids. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent, one portion was treated with equal amount of Dragondroffs' reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The appearance of creamish precipitate, the orange precipitate and brown precipitate indicated the presence of respective alkaloids .

Test for saponins

About 0.5 g of the plant tuber extract was vigorously shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponins.



Test for tannins

About 0.5 g of plant tuber extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

Test for steroids

2 ml of acetic anhydride was added to 2 ml of plant tuber extract of each sample along with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoids and orange colour for flavones.

Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids.

Test for Proteins

To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% CuSO₄ solution was added. A violet colour indicated the presence of peptide linkage of the molecule

Test for Amino Acids

To 2 ml of sample was added to 2 ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple colour indicated the presence of amino acids in the sample

Test for Tri-Terpenoids

5ml of each extract was added to 2ml of chloroform and 3ml of con. H₂SO₄ to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids.

Test for Reducing Sugar

To 2 ml of extract 2drops of Molisch's reagent was added and shaken well. 2ml of conc. H₂SO₄ was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

RESULTS AND DISCUSSION

Table 1: Preliminary Phytochemical analysis of Aqueous, Chloroform and Methanol extract of *Nalla marunthu*

S.No	Phytochemical Constituents	Aqueous extract	Chloroformic extract	Methanolic extract
1	Flavanoids	++	++	--
2	Alkaloids	--	++	++
3	Tri-Terpenoids	--	--	--
4	Saponins	--	--	++
5	Tannins	--	++	++
6	Reducing Sugars	++	++	--
7	Amino Acids	++	--	--
8	Proteins	++	--	--
9	Anthroquinones	--	--	++
10	Steroids	++	--	++
11	Cardiac Glycosides	--	++	++

Medicinal plants play a vital role in the health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various human ailments because they contain the components of therapeutic value (13).. In addition, plant based drugs remain an important source of therapeutic agents because of the availability, relatively cheaper cost and non-toxic nature when compared to modern medicine . Many herbs contain antioxidant compounds which protects the cells against the damaging effects of reactive oxygen species (14) .Table 1. showed that the phytochemical constituents of aqueous, methanolic and chloroformic extracts of *Nalla marunthu* were screened. The phytochemical screening of the crude extracts of aqueous extract revealed the presence of Flavonoids, Tannins, Tri-terpenoids, Cardiac glycosides and Steroids remaining all are absent. In methanol extract of herbal formulation contains Flavonoids, Tannins, Steroids, Anthroquinines and Gardiac glycosides and remaining phytoconstituents was absent. Flavonoids, Alkaloids, Triple sugars , Proteins and Amino acids were present in the chloroform extract of *Nalla marunthu* and remaining phytochemicals were absent.

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