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Original Article

Screening of Phytochemical Analysis and *In vitro* Bioactive of Polyherbal Formulation

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ABSTRACT

Introduction: Plants have the capability to synthesize various forms of phytochemical compounds as secondary metabolites. **Method**: In the present investigation phytochemicals such as alkaloids, tannins, glycosides, carbohydrates, reducing sugar, proteins, saponins, flavanoids, phenols, terpenoids and phytosterols were detected in polyherbal formulation *A. marmelos, G. glabra* and *R. centrifolia*. **Results**: The Total flavonoid, steroids, alkaloids and phenolic content was observed from fruit, root and pettles1.40mg QE/g DE, 12.14mg BE/g DE, 14.40mg AE/g DE and 99.33mg GAE/g DE. FTIR spectrum of the polyherbal sample revealed 5 major peaks at 2919.40 cm^{-1}, 2357.62 cm^{-1}, 1150.56 cm^{-1}, 1076.22 cm^{-1} and 1015.64 cm^{-1}. The antibacterial activity was maximum zone of inhibition (19 mm) was recorded in *S. aureus* strain and minimum zone of inhibition (5mm) was observed in *S.mutans* strain. The antioxidant study maximum and minimum scavenging DPPH, NOR, H2O2 and SOD activities (%) of 62.28, 53.68, 39.67 & 43.98 at 5 mg/ml and 39.88, 36.49, 5.9 and 34.94 at 1mg/ml was recorded. The polyherbal sample exhibited significant albumin denaturation, proteinase inhibitory, membrane stabilization and lipid peroxidation inhibitory activities as the maximum inhibition of 46.53%, 36.7%, 51.9% and 64.71% was observed at 500 µg/ml. **Conclusion:** Hence the present studies indicate good antibacterial, antioxidant and anti-inflammatory activities from the medicinal plants, *A. marmelos, G. glabra* and *R. centrefolia* proves the possibility of its utilization as an additional potent source medicinal uses.

Key words: Phyto Chemical, Secondary Metabolites, FTIR, HR-LCMS, Antimicrobial Activity, Antioxidant Activity.

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INTRODUCTION

The finding of new molecules can contribute to the development of basic scientific concepts, lead to valuable drug oriented compounds and suggest possible new pharmacological reagents. The high demand for medicinal plants is associated with their physicochemical properties and biological activities and has led to the emergence of phyto-chemicals as commercially important compounds that have found usefulness in food, cosmetics, and pharmaceutical industries. Secondary metabolites often have unique biological actions and thus deserve special attention. In addition, some compounds from plant parts show significant biological activity at an extremely low concentration. Medicinal plants have been used in traditional medicine in meeting the health needs of people in India. In India, research emphasizing discovery of medicinal plants with antimicrobial, antioxidant, anti-inflammatory and anticancer activities is ongoing and recognition has been given to the application of medicinal plants in the treatment and management of several diseases, including heart diseases. The fruit is also diuretic, being effective in the treatment of dropsy and renal stones. The root is purgative and in large dose is said to be emetic[1]. Antimicrobial polyherbal (AMP) are important components of the host innate immune response against microbial invasion. AMP is usually characterized by their small-size, heat-stability and broad range of antimicrobial activity.Bioantioxidants are necessary components of all tissue and cells of living organisms, where their normal physiological concentrations maintain free-radical autooxidation processes at a low stationary level[2]. Plant polyerbal product anti-inflammatory compounds are the practical examples of these medications which are associated with adverse effects while in practice our purpose is to apply minimum effective dose by the highest efficiency with the least adverse effects[3]. In present study was designed to examine the total polyherbal formulation and phytochemical analysis of aqueous extract of fruit, root and pettles of *A. marmelos, G. glabra* and *R. centerifolia* were screened for antimicrobial, antioxidant and anti-inflammatory properties using standard methods. The findings from this work may add to the overall value of the medicinal potential of the plants.

MATERIALS AND METHODS

The plant was collected in January 2018 from Rumi herbals pvt ltd. The plant was identified by their common names *Aegle marmelos* (Common Name - Bael); (Family – Rutaceae); (Part – fruit), *Glycyrrihza glabra* (Common Name - Liquorice); (Family – Fabaceae); (Part – roots), *Rosa centrifolia* (Common Name - White rose); (Family – Rosaceae); (Part – pettles) and later it was compared with the herbarium of the Department of Studies in Botany, pachaiyappa's college tamilnadu, Chennai.

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Aqueous extract

Initially, distilled water (Aq) (100 mL) was added to the polyherbal formulation material (40 g), the mixture was maintained on a rotary incubator (220 rpm, 24 h, 22 °C). After this period, the mixture was filtered using Whatman nº 1 filter paper and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected giving rise to the aqueous extracts, it was maintained at a temperature of 4 º C until used in the bioassays.

Phytochemical screening

Aqueous extracts of polyherbal formulation *A. marmelos, G. glabra* and *R. centrifolia* were tested for the presence of alkaloids, tannins, glycosides, carbohydrates, reducing sugar, proteins, saponins, flavanoids, phenols, terpenoids and phytosterols according to a methodology [4].The qualitative results were expressed as presence/ positive reaction (+) weakly positive and absence /negative reaction (-) of phytochemicals.

Determination of total flavonoid contents

The total flavonoïd contents of the polyherbal formulation extracts were quantified by spectrophotometric (Thermo Fisher Scientific, Genesys, Madison, USA) measurement of the absorbance according to the FolinCiocalteu, using the aluminum chloride and by the vanillin methods[5]

Determination of steroids contents

In this test acetic anhydride (2 ml) was mixed to 0.5 g of each crude extracts of polyherbal formulation *A. marmelos, G. glabra* and *R. centrifolia* followed by adding of 2 ml of H_2SO_4. The color changed from violet to green or blue in some samples authenticates the presence of steroids.[6]

Determination of total alkaloids

Exactly 1g of the polyherbal sample was weighed into a 250 ml beaker and 40 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. The whole solution was allowed to settle and the precipitate was collect washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and quantified.[6]

Determination of total phenolic content

Total phenolic content (TPC) in extracts was determined by Folin-Ciocalteu's colorimetric method.[7]

Fourier Transform Infra Red (FT-IR) analysis

FT-IR spectrum of crude extract was taken relied on a Bio-Rad FT-IR-40 model, USA. Sample (10 µg) was mixed with 100mg of dried KBr and compressed to prepare as a salt disc (10 mm dm) for reading the spectrum further.

HR-LCMS

The total content of phyto-chemicals in extracts was separation according to the method. Mass spectrometric analysis was performed on a Shimadzu mass spectrometer.

Antimicrobial activity

Plant polyherbal crude extract was tested for inhibition against the human pathogenic bacteria. Microbial assay were carried out by well diffusion technique.[8]

Antioxidant Activities

Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH)

The scavenging activity of the DPPH free-radical was assayed according to the method.[9]

NO Reduction assay

The NO Reduction assayof polyherbal was assessed by the modified method.[10]

Hydroxyl radical scavenging activity

The scavenging activity of polyherbal against the hydroxyl radical was investigated using Fenton's reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$). Hydroxyl radicals were generated using a modified.[11]

Superoxide radical scavenging activity

The superoxide radical scavenging abilities of polyherbal were assessed by the modified method.[12]

Anti-inflammatory activity

Preparation of Raw 246.7 macrophages cell suspension

A subculture of Raw 246.7 macrophages in Dulbecco's Modified Eagle's Medium (DMEM) was trypsinized separately, after discarding the culture medium. To the disaggregated cells in the flask 25 mL of DMEM with 10% FCS was added. The cells suspended in the specific medium by gentle passage with the pipette and the cells homogenized.

Seeding of cells

One mL of the homogenized cell suspension was added to each well of a 24 well culture plate along with different concentration of sample AM dry extract (0 to 400 µg/mL) and incubated at 37°C in a humidified CO2 incubator with 5% CO2. After 48 hrs incubation the cells were observed under an inverted tissue culture microscope. With 80% confluence of cells cytotoxic assay was carried out.

Cytotoxicity assay

The assay was carried out using (3-(4, 5-dimethyl thiazol-2yl)-2, 5- diphenyl tetrazolium bromide (MTT). MTT assay is cleaved by mitochondrial Succinate dehydrogenase and reductase of viable cells, yielding a quantifiable purple product formazan. All wells were removed the content using pipette and 100µl SDS in DMSO were added to dissolve the formazan crystals, absorbance's were read in Lark LIPR-9608 micro plate reader at 540 nm.[13]

Inhibition of nitric oxide (NO) production

Cell culture

The RAW 264.7 macrophage cell lines were cultured in plastic culture flasks in Dulbecco's Modified Eagle's Medium containing l-glutamine supplemented with 10 % foetal calf serum (FCS) and 1 % antibiotic solution (Gibco, USA) solution under 5 %CO2 at 37 °C, and were split twice a week. Cells were seeded in 96 well-microtitre plates and were activated by incubation in medium containing LPS (5 µg/mL) and various concentrations of extracts dissolved sterile DMEM medium.[14]

Measurement of nitrite

Nitric oxide released from macrophages was assessed by the determination of nitrite concentration in culture supernatant using the Griess reagents. The absorbance of the resultant solutions in the wells of the microtitre plate was determined with a microtitre plate reader (Readwel touch, Micro plate Reader, India) after 10 min at 550 nm.

Albumin Denaturation Assay (ADA)

The albumin denaturation assay of polyherbal was studied by using inhibition of albumin denaturation technique which was studied.[15]

Proteinase Inhibitory Activity (PIA)

The proteinase inhibitory activity test was performed according to the modified method.[16]

HRC Membrane Stabilization Activity (HRC)

The membrane stabilization activity test was performed according to the modified method.[17]

Lipid peroxidation Activity (LPA)

The lipid peroxidation activity of polyherbal was assessed by the standard method.[18]

RESULTS

Phytochemical screening

Alkaloids, tannins, glycosides, carbohydrates, reducing sugar, proteins, saponins, flavanoids, phenols, terpenoids and phytosterols were detected (Table 1).

Determination of total flavonoid, steroids, alkaloids and phenolic contents

The Total flavonoid, steroids, alkaloids and phenolic content was observed in polyherbal formulation *A. marmelos, G. glabra* and *R. centrifolia* from fruit, root and pettles1.40mg QE/g DE, 12.14mg BE/g DE, 14.40mg AE/g DE and 99.33mg GAE/g DE.

FTIR spectral analysis

FTIR spectrum of the polyherbal sample revealed 5 major peaks at 2919.40 cm^{-1}, 2357.62 cm^{-1}, 1150.56 cm^{-1}, 1076.22 cm^{-1} and 1015.64 $cm^{-1}$and the remaining peaks are closely lying between 1015.64 cm^{-1} and 3851.67 cm^{-1} (Figure 1).The signals at 2919.40 cm−1 correspond to C-H stretch in primary and secondary carboxylic bands. The FT-IR spectrum of the polyherbal the ester band started from 1150.56cm^{-1} respectively. The anhydrides group is represented by a band in 1076.22cm^{-1} and 1015.64cm^{-1} the carbon group at 1653 cm^{-1}.

Liquid chromatography coupled to mass spectrometry (HR LC/MS)

The present study is the first to investigate and identify phytochemical compounds of polyherbal extract from *A. marmelos, G. glabra* and *R. centrifolia* (Figure 2). The predominant compounds of fruit, root and pettles .Norcotinine (Rt=0.798 min) at m/z=162,Lupanyl acid (Rt=0.813 min) at m/z=252,Agmatine (Rt=0.826 min) at m/z=130, Lactulose (Rt=0.955 min) at m/z=342,Tranexamic acid (Rt=1.088 min) at m/z=157,Nomifensin (Rt=4.006 min) at m/z=238,Piin (Rt=5.883min) at m/z=564,Hoifolin (Rt=6.333min) at m/z=578,Phenylpropiolic acid (Rt=6.673min) at m/z=146,Citropten (Rt=6.673min) at m/z=146, Gummiferol (Rt=8.187min) at m/z=286, Genkwanin (Rt=8.572min) at m/z=284,Formononetin (Rt=10.3min) at m/z=268,Rifampin (Rt=10.83min) at m/z=822 and Crotamiton (Rt=12.713min) at m/z=203.

Antimicrobial activity

The zone of inhibition in different bacterial strains against polyherbal extraction is shown in (Figure 3 and Plate I). Among the various bacterial strains maximum zone of inhibition (19 mm) was recorded in *Staphylococcus aureus* strain and minimum zone of inhibition (5mm) was observed in *Streptococcus mutans* strain.

Antioxidant Activities

In the present study maximum and minimum scavenging (DPPH) activity (%) of 62.28 at 5 mg/ml and 39.88 at 1mg/ml was recorded in the polyherbal sample (Figure 4). The maximum and minimum NO reduction ability (%) of 53.68 at 5 mg/ml and 36.49 at 1 mg/ml was reported in polyherbal sample. The maximum and minimum scavenging (H2O2) activity (%) of 39.67 at 5 mg/ml and 5.9 at 1 mg/ml was reported in polyherbal sample. The maximum and minimum scavenging (SOD) activity (%) of 43.98 at 5 mg/ml and 34.94 at 1 mg/ml was reported in polyherbal sample. The antioxidant activity was comparable with standard ascorbic acid.

Anti-inflammation assay

The *in-vitro* cytotoxicity activity results of the polyherbal extract sample against Raw246.7 macrophages cells were triggered of cytotoxicity significantly with the increasing of sample concentration and it was observed. In this cell lines, cytotoxicity assay was observed in tested sample concentrations in 48 hours treatment, it also observed that increased concentration of test samples shown increased cytotoxicity over the tested cell lines (Figure 5). It was evident that the less cytotoxicity of the test sample showed no cell disintegration and migration after 48 h of treatment against the selected tested cell lines effect. It was calculated that the IC50 of the test sample AM dry extract against Raw 246.7 macrophages cells was 326.274 µg/ml.

The inhibitory activity of the polyherbal extract on NO production by induced RAW264.7 macrophage cell lines. Sample polyherbal dry extract had the best inhibitory activity on NO production (32.79 % inhibition / 75.51 % cell viability) at 12.5µg/mL (Figure 6).

Table 1: Phytochemical prospection of aqueous extracts from polyherbal samples.

S.No	Screening	A. marmelos	G. glabra	R. centifolia	Vlco1
1	Alkaloids	+	+	-	+
2	Tannins	+	+	-	+
3	Glycosides	+	+	+	+
4	Carbohydrates	+	+	-	+
5	Reducing sugar	+	+	+	+
6	Proteins	+	+	+	+
7	Saponins	-	+	+	+
8	Flavanoids	+	+	+	+
9	Phenols	+	+	-	+
10	Terpenoids	+	-	-	+
11	Phytosterols	+	+	+	+

+ Positive reaction – Negative reaction

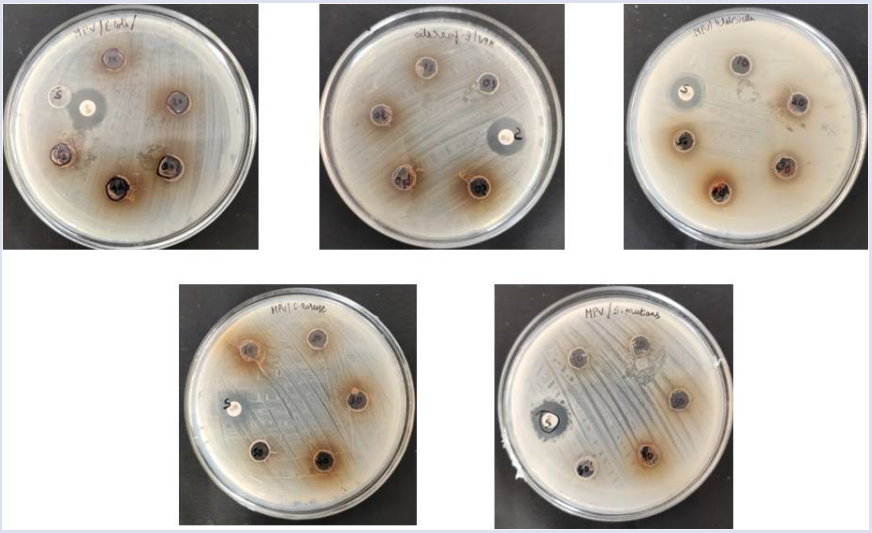

Plate 1: Antimicrobial activity of crude extract.

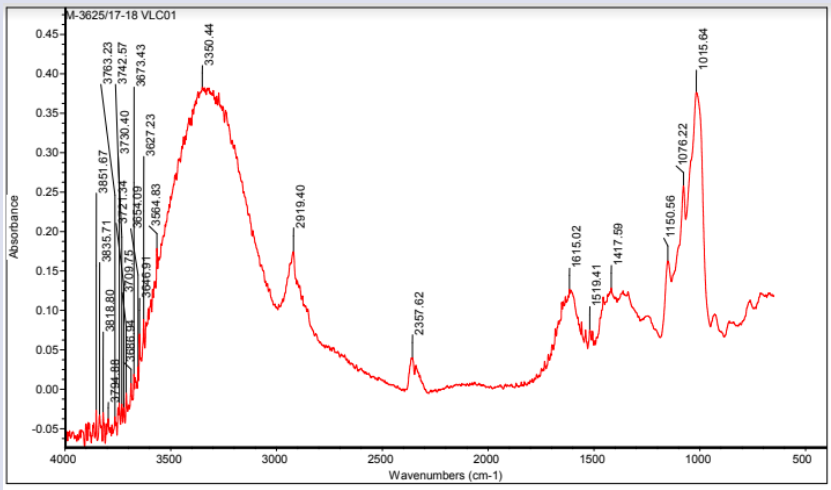

Figure 1: FTIR spectrum of polyherbal sample.

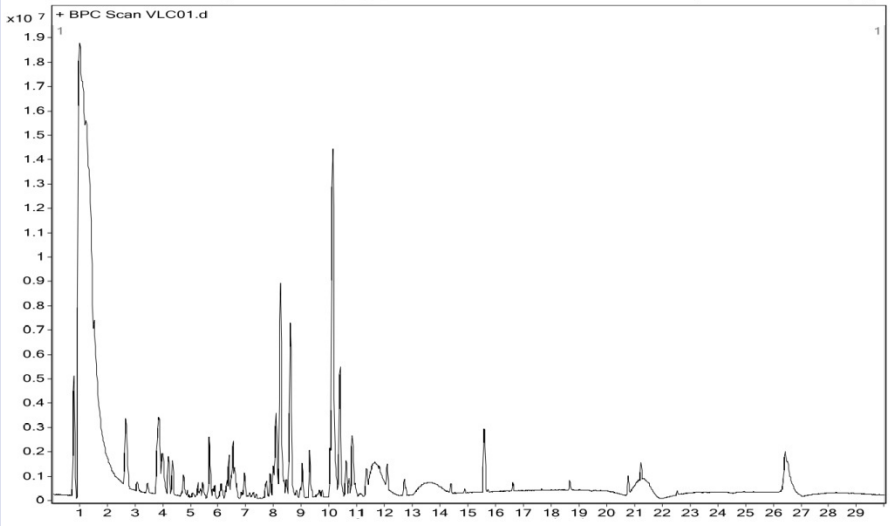

Figure 2: LC/MS characteristics of phytochemical compounds. Compounds listed in order of retention time; Rt= retention time (as min).

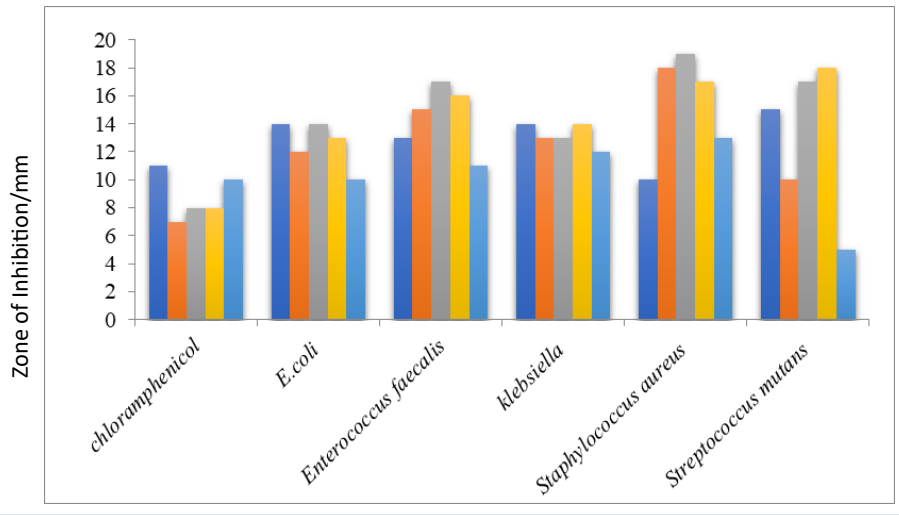

Figure 3: Antimicrobial activity of crude extract.

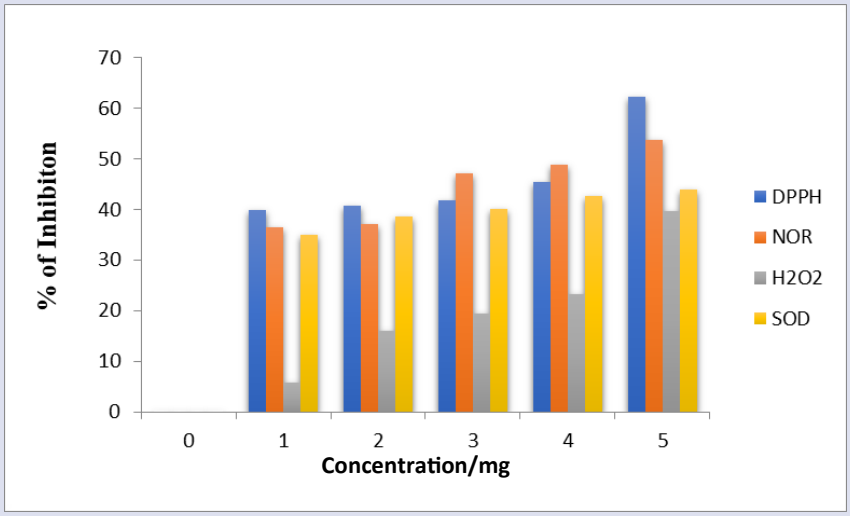

Figure 4: Scavenging activity of polyherbal sample.

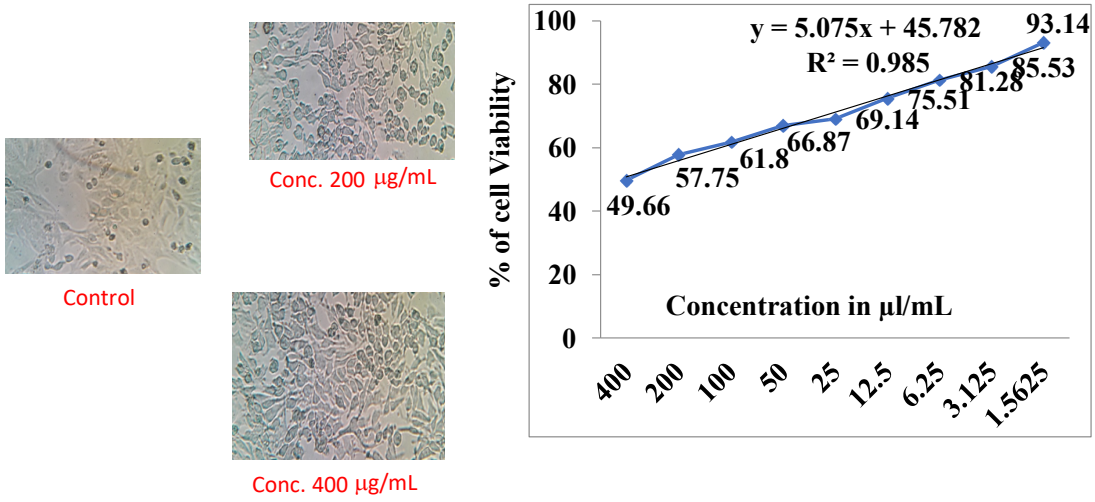

Figure 5: Cytotoxicity effect of sample against Raw 246.7macrophages Cell lines.

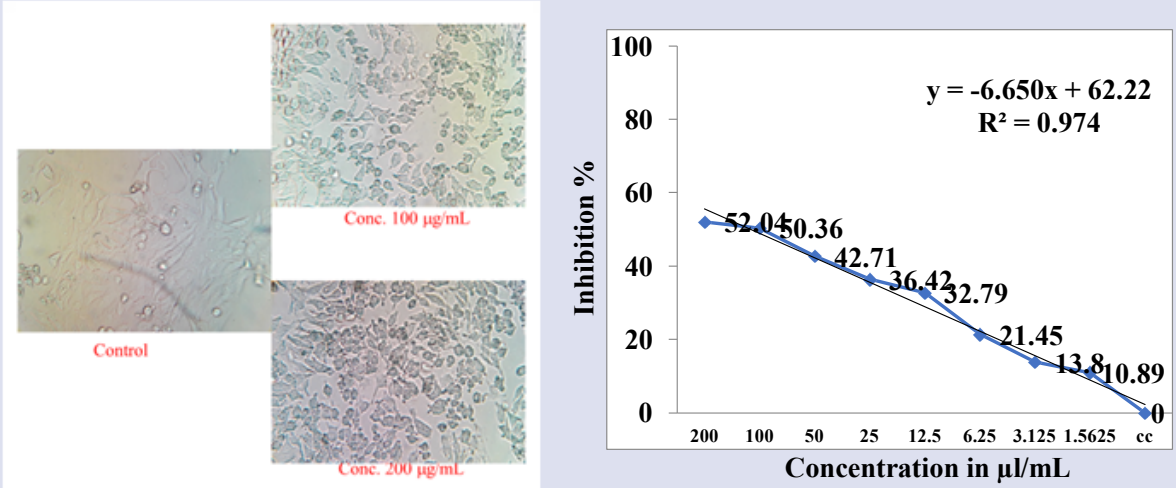

Figure 6: Cytotoxicity effect of sample against Raw 246.7 macrophages Cell lines.

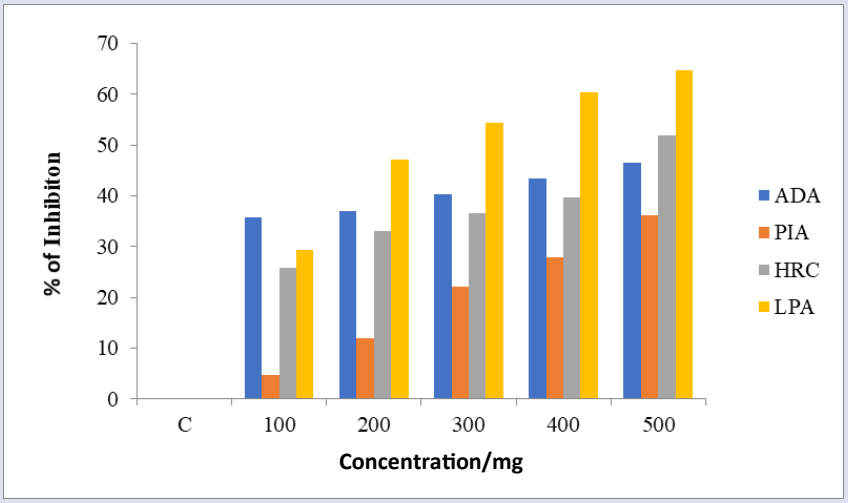

Figure 7: Effect of aqueous extracts of polyherbal on albumin denaturation, proteinase inhibitory, membrane stabilization and lipid peroxidation inhibitory activities.

In the present investigation the polyherbal sample exhibited significant albumin denaturation, proteinase inhibitory, membrane stabilization and lipid peroxidation inhibitory activities at different concentrations as determined. Maximum inhibition of 46.53%, 36.7%, 51.9% and 64.71% was observed at 500 µg/ml (Figure 7).

DISCUSSION

In India, medicinal plants phytochemicals are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. Plants are considered as potent source of biological active compounds that are able to produce different important secondary metabolites described with great biological activities. In the past few decades several investigations are observed that the plants polyherbal product and the phyto-chemicals derivatives have received the most attention of researchers. In present study was designed to evaluated the total polyherbal formulation and phytochemical analysis of aqueous extract of fruit, root and pettles of *A. marmelos, G. glabra* and *R. centrifolia* were screened for antimicrobial, antioxidant and anti-inflammatory properties using standard methods.

In the present investigation determination of phytochemicals such as alkaloids, tannins, glycosides, carbohydrates, reducing sugar, proteins, saponins, flavanoids, phenols, terpenoids and phytosterols were observed. Phytochemicals present in different parts of the plants, such as in the root, stem, leaf, flower, fruit and seed. Phyto- chemicals has long been documented that fruits and vegetables are essential toa healthy and well balanced diet required for healthy living and that high consumption is beneficial to health in combating the onset of cancer, coronary disease, inflammation, arthritis, antibacterial compounds, antioxidant, immune system decline, brain dysfunction and cataracts. The aqueous extract all Polyherbal presented positive reaction (+) for the presence of phytochemicals.

The aqueous extract was the only one that presented flavanonols, which are considered partially polar flavonoids and moderately soluble in polar solvents, such as water[19]. Saponins were found only in the aqueous extract and it is known that saponins have high polarity. The phenolic compounds, condensed tannins were detected when more polar solvents were used, such as ethanol, methanol and water [20].The different kinds of secondary metabolites detected in *A. marmelos, G. glabra* and *R. centrifolia* leaves extracts, in the present work, are in

agreement with the reported in the previous literature for other species of the same class. Polyherbal determined with phytochemical reported the presence of steroids alkaloids, tannins, glycosides, carbohydrates, reducing sugar, proteins, saponins, flavanoids, phenols, terpenoids and phytosterols [21]which may be related to the biological activities found in the present studies.

In the present evaluation the FTIR spectrum of the polyherbal sample revealed 5 major peaks at 2919.40 cm^{-1}, 2357.62 cm^{-1}, 1150.56 cm^{-1}, 1076.22 cm^{-1} and 1015.64 $cm^{-1}$and the remaining peaks are closely lying between 1015.64 cm^{-1} and 3851.67 cm^{-1}.The signals at 2919.40 cm−1 correspond to C-H stretch in primary and secondary carboxylic bands. The FT-IR spectrum of the polyherbal the ester band started from 1150.56cm^{-1} respectively. The anhydrides group is represented by a band in 1076.22cm^{-1} and 1015.64cm^{-1} the carbon group at 1653 cm^{-1} [22] was probably the first researcher to suggest the possibility of using infrared spectroscopy for biological sample. The plants phytochemical compounds are different in molecular structure and characterization. Analysis of the FT-IR spectrum showed typical absorption bands corresponding to N-H stretching of proteins and peptide bonds, giving solid evidence that the substance contained a peptide in its structure. Again the FT-IR spectral analysis of the polyherbal phytochemical from *A. marmelos, G. glabra* and *R. centerifolia* showed more or less same number of peaks, lying within the same range of values of the commercial phytochemical used as a standard.

The present study is the predominant compounds of fruit, root and pettles Norcotinine(Rt=0.798 min) at m/z=162,Lupanyl acid (Rt=0.813 min) at m/z=252,Agmatine (Rt=0.826 min) at m/z=130, Lactulose (Rt=0.955 min) at m/z=342, Rifampin (Rt=10.83 min) at m/z=822 and Crotamiton (Rt=12.713 min) at m/z=203 reported [10]that the accumulate compounds of *E. glaucophyllum* flowers extract were: quinic acid (Rt=1.7 min) at m/z=191, rutin (Rt=21.8 min) at m/z=609, gallic acid (Rt=2.8 min) at m/z=169 and Apegenin-7-o-glucoside (Rt=28.4 min) at m/z=431.The phytochemical characteristics of *A. marmelos, G. glabra* and *R. centerifolia* extract proved to be very similar to plants.

The use of medicinal plants plays a crucial role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.[10] In the present investigation the zone of inhibition in different bacterial strains the maximum zone of inhibition (19 mm) was recorded in *S. aureus* strain and minimum zone of inhibition (5 mm) was observed in *S. mutans*strain from *A. marmelos, G. glabra* and *R. centerifolia*.

Generally, plants extracts are usually more active against gram positive bacteria than gram negative bacteria. According[25]gram negative bacteria are more resistant to plants extract compared to gram positive bacteria. This may be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism. This is in support of the present finding which showed that only polyherbal phytochemical from *A. marmelos, G. glabra* and *R. centerifolia* was effective on the growth of *S.aureus*. The positive control (chloramphenicol) was showed activity against the bacterial strains tested and the maximum activity 11 mm. In addition, ethanol extracts of this herb have been reported to be effective against a-Streptococcus group[26]. The use of fruit, leave and root agents for medicinal benefits has played an important role in anti-bacterial agents derived from terrestrial plants. The polyherbal phytochemical from *A. marmelos, G. glabra* and *R. centerifolia* the aqueous extracts showed significant antimicrobial activity when compared with control (antibiotic chloramphenicol).

Plants antioxidant agents like phenolic acids, polyphenols and flavonoids are capable to scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanism that lead to degenerative diseases. In the present study maximum and minimum scavenging (DPPH, NOR, H2O2 and SOD) activities (%) of 62.28, 53.68, 39.67 & 43.98 at 5 mg/ml and 39.88, 36.49, 5.9 and 34.94 at 1mg/ml was recorded in the polyherbal sample. In recent years, there has been an increasing interest in finding natural antioxidants, since they can protect advancement of many chronic diseases.[27] The antioxidant activity of phytochemical may be attributed to various mechanisms, which includes prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reductive capacity and radical scavenging. The scavenging ability of the *E. glaucophyllum* extracts samples (leaves and flowers) on DPPH free radical was determined 25.2 ± 1.9% to 91.9 ± 0.1%[10]. The effect of antioxidants on DPPH radical scavenging was due to their hydrogen donating power. The present results indicate that the phytochemical was better hydroxyl radical scavenger. Antioxidants of 0.04, 0.31, 0.58, 2.30 and 0.054 mg/ml were recorded for *Psidium guajava*, *Magniferaindica*, *Caricapapaya*, *Vernonia amygdalina* and Vitamin C. When the results are compared, the polyherbal had stronger scavenging activity for superoxide radical than control. The mechanism of these results is that the inner structure of phytochemical is severely disrupted by the introduction of grafted polymer chains after modification. The ability to form hydrogen bond declines sharply and the hydroxyl and sulfate groups are activated, so this is helpful to the reaction with superoxide anion..

Albumin denaturation and proteinase inhibitory was effective in the maximum inhibition of 71% and 53%was observed at 500 µg/ml reported[28]. The *Wedeliatrilobata* leaf and stem ethanol extract also showed anti-inflammatory activity by inhibiting the heat induced albumin denaturation and red blood cells membrane stabilization with 87.14 and 86.76 and 78.11, 74.17 g/ml respectively [29]In the present investigation the polyherbal sample exhibited significant albumin denaturation, proteinase inhibitory, membrane stabilization and lipid peroxidation inhibitory activities at different concentrations as determined. Maximum inhibition of 46.53%, 36.7%, 51.9% and 64.71% was observed at 500 µg/ml. All doses of the extract tested were effective in reducing polyherbal throughout the experiment. The present concluded that aqueous extract of the plant parts had appreciable anti-inflammatory activity and thus justifies its use in traditional medicine in the treatment of inflammatory diseases.

In the present investigation the *in-vitro* cytotoxicity activity results of the polyherbal dry extract sample was calculated that the IC50 of the test sample polyherbal dry extract against Raw 246.7macrophages cells was 326.274 µg/ml. The inhibitory activity of the AM dry extract on NO production by induced RAW264.7 macrophage cell lines is the best inhibitory activity on NO production (32.79 % inhibition / 75.51 % cell viability) at 12.5µg/mL. Sample AM dry extract with good inhibitory activity on NO production and a low cytotoxicity are more useful. Release of NO promotes inflammation, therefore extracts that could act as scavengers of NO, or inhibitors of its production, especially with corresponding low cytotoxicity could be used to mitigate the propagation of inflammation by NO.[30] Showed a maximum of 46.86% oedema inhibition at 3 h. at the dose of 200 mg/kg and the anti-inflammatory effect tested for 3 h. for *Glycyrrhiza glabra*. Anti-inflammatory study showed that the extracts of *Terminaliabellarica* (73.34 %) and *T. chebula* (74.81 %) showed significant COX-2 selective inhibition as compared to other samples. In the present report the cytotoxicity determination study of aquouse fraction of selected medicinal plants indicates that the selected samples have no effect on cell viability. Present findings provide technical evidence to support phytochemicals medicinal uses and indicate a promising potential for the development of an anti-inflammatory agent from *A. marmelos, G. glabra* and *R. centrifolia* plants.

Plants have the capability to synthesize various forms of phytochemical compounds such as primary and secondary metabolites, many of which have been identified and continue to be relevant in the treatment of

complicated disease conditions in the Indian setting. Interestingly, researcher have examined some of these Indian medicinal plants and acknowledged their biological and therapeutic activities. Hence the present studies good antibacterial, antioxidant and anti-inflammatory activities from the medicinal plants, *A. marmelos, G. glabra* and *R.centrifolia*proves the possibility of its utilization as an additional potent source medicinal use.

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CONFLICTS OF INTEREST

None.

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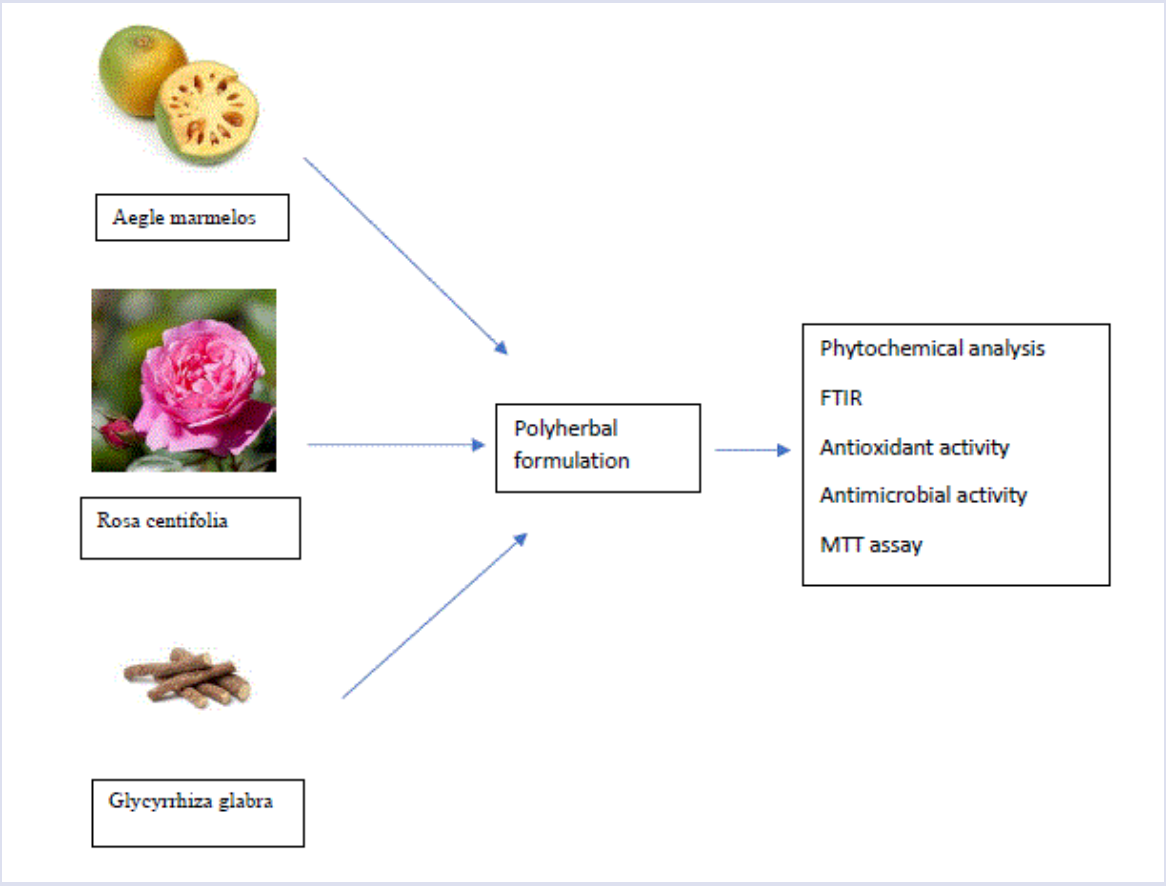
GRAPHICAL ABSTRACT



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