Phytochemical Screening, Hptlc, Antimicrobial Activity In Extracts Of Psidium Guajava And Piper Betle Leaves Combination (PGPB)

K. Sudha Rameshwari, R. Sathiya Priya

Abstract: The present work was carried out to assess the leaves of Psidium guajava and Piper betle in combination (PGPB) for their antimicrobial activity against human bacterial pathogens. The extracts were prepared using methanol, aqueous and ethyl acetate using cold extraction methods. Flavonoids, tannins, phenols, terpenoids, carbohydrates, saponins, alkaloids, cardiac glycosides, proteins, sterols, tri terpenoids were present in methanol and ethyl acetate extract except aqueous extract. Fixed oils are absent in all extract. The activity of plant extract was tested against Micrococcus sp., Streptococcus sp, Neisseria sp., Vibrio sp., Shigella sp., Pseudomonas sp., E.coli, Bacillus subtilis through agar well diffusion method. Methanol and aqueous extract has more significant antimicrobial activity against selected pathogens than ethyl acetate extract. Methanol extract showed extremely significant activity against Shigella sp. compared to standard antibiotic ampicillin. High performance thin layer chromatography (HPTLC) is a planar chromatography where separation of sample components is achieved on high performance layers with detection and data acquisition. The phyto constituents (flavanoids, steroids, triterpens) present in the methanol and aqueous extract was further confirmed by HPTLC. In methanol extract has more bands (many constituents) than aqueous extract. The antimicrobial activity of leaves of PGPB shows the presence of broad spectrum of antimicrobial compounds which act against gram positive and gram negative bacteria especially in methanol, aqueous and ethyl acetate. Further, isolation, purification of bioconstituents from the methanol extracts leads to the development of new antimicrobial drug and also promotes the development of anti-rheumatic drug.

Index Terms: antimicrobial activity, HPTLC, Psidium guajava, Piper betle, anti-rheumatic drug

1. INTRODUCTION

Immune device of our body performs a important function, as an overactive immune gadget may also cause certain fatal disease because of diverse hypersensitive or hypersensitive reactions which can also purpose numerous derangements; lack of everyday potential to distinguish self from non-self ensuing in immune reactions in opposition to our very owns cells and tissues called autoimmune diseases. Rheumatoid arthritis (RA) is one of the auto immune diseases. Already they have swollen pain in all joints due to inflammation. The RA patients were very poor in immune system. In addition to this, they can easily suffer with infections due to many microorganisms. To overcome this problem, it is necessary to develop a drug without side effects. Environment has provided us many riches of medicinal plants as a source of healing agents for the avoidance and the treatment of many diseases. One of such medicinal plants is Psidium guajava and Piper betle. However, to our knowledge, no data are available regarding anti-rheumatic efficacy of two medicinal plants combine (leaves of Psidium guajava and Piper betle). The coronary heart fashioned Betel leaf is beckoned as the ‘Golden heart of nature’. The Chinese language conventional medication also used Betel leaves for its warm and highly spiced nature that aided within the treatment of cough, itching, infection, headache and respiration infections.

Betel leaves had been used in diverse scientific preparations of the Unani remedy and become used as a brain tonic, and in treating throat infections, cleaning the blood and for boosting the urge for food. Psidium guajava acclaimed as the “poor man's apple of the tropic”. It has been used in the treatment of diarrhea, dysentery, menstrual issues, vertigo, anorexia, digestive issues, gastric insufficiency, infected mucous membrane, laryngitis, pores and skin troubles, ulcers, vaginal discharge, bloodless, cough, cerebral ailments, nephritis, jaundice, diabetes, malaria and rheumatism to mention a few. The present study was undertaken to assess the anti-microbial activity of two medicinal plants combined (leaves of Psidium guajava and Piper betle).

2. MATERIALS AND METHODS

2.1 Collection of samples
Psidium guajava and Piper betle leaves are collected from the local area of Virudhunagar, Tamilnadu, India. The cultures are collected from Microbiology department, V.V. Vanniaperumal College for women, Virudhunagar, Tamilnadu, India. All these cultures were maintained on Luria Bertani broth at 4°C in lab.

2.2 Preparation of plant extracts:
The leaves of Psidium guajava and Piper betle were shade dried and they were minced individually using a mixer. The individual plant leaves. The dried powder was stored in an air-tight container at room temperature until further use. The powdered plant material (20g) was sequentially extracted three times with 200ml of ethyl acetate, methanol and water at room temperature for 48 hours. The extracts were filtered through muslin cloth and air dried. The dried extracts were transferred to sample bottles which were placed in a desiccator containing anhydrous calcium carbonate and

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Date of submission: 24.01.2020
small amount of silica gel to remove any traces of water that could have been present. The dry extracts were later kept in tightly stoppered bottles in a refrigerator for further analysis.

2.3 Preliminary qualitative phytochemical analysis:
One gram of plant extracts was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% w/v and tested for the presence of carbohydrates, proteins, sterols, alkaloids, tannins, glycosides, flavonoids, phenolic compounds, and saponins [1].

2.4 Evaluating the antimicrobial activity of crude plant extracts
The test micro organism was aseptically inoculated on sterile Mueller Hinton agar by surface spreading to make uniform microbial inoculums. Using sterile glass cork borer, wells were carefully made on the agar plate without distorting the media; wells contain the test extract, the control and standard drug; Ampicillin (10 mg/ml) and individual solvent was used as a negative control (Standard) against microorganisms. Fifty microlitres (50 μl) of the extract and the controls(respective solvents) were carefully dispensed into the respective wells and the plates left on the bench for 60 minutes to allow the system stabilize as the inoculated microorganisms get acclimatized to the new environment. The culture plates were then incubated at 370C for 24 hours. Using a metric ruler, the diameter of the zone of inhibition was measured in mm.

2.5 Characterization of extract:
Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe best the physicochemical parameters. Consequently the cutting-edge strategies describing the identity and quantification of active components within the plant fabric may be beneficial for proper standardization of herbals and its formulations. Also, the WHO has emphasized the want to make certain the exceptional of medicinal plant products the use of present day managed techniques and applying suitable standards.

2.5.1 High performance thin layer chromatography (HPTLC):
Silica gel 60F 254 HPTLC plates in the format of 4x10cm are used. Develop plate with 20ml of mobile phase per trough in a 4x10cm.Twin trough chamber to the upper edge equilibrate plate with lab atmosphere in a suitable container providing protection from dust and fumes. Plates are handled either on both sides or on the top edges. 2μl samples are applied as 8mm bands using a suitable instrument (Linomat 5). Developing solvents consisting of more than one component are prepared by measuring the required. The appropriate volume of developing solvent (4x10cm) was prepared. The chamber was opened and placed the correctly sized piece filter paper in the rear trough. The solvent was poured into chamber so that filter paper was wetted and adheres to rear wall of TLC. The different solvent system were used for identification and separation of different phytoconstituents (n- Butanol: Acetic acid: Water.(4:1:1) for Triterpenes and Flavonoids; Toluene: Et. Acetate: Methanol (7:2:1) for steroids).The chamber was tilted to the side so that the solvent volume in both troughs equalizes. The chamber was equilibrated for 2 hours. The plate was inserted into the front trough. The layer faces the filter paper and the back of the plate rests against front wall of TLC. The lid was replaced and develops plate to the mark. The plate was removed and dried it for5minutes in a steam of cold air. Prior to preparation for the next run the chamber is dried and cleaned. Transfer of reagents for derivation of samples on a HPTLC plate may be accomplished by spraying (Derivatization @520nm- For Triterpenes : Liebermann-Burchard regent and heat the plate at 105 o C for 5 min, Shows Blue, green, pink, brown, yellow in visible light also under UV light. For Flavonoids: Aluminium chloride Spray solution: Yellow fluorescence in UV light. For Steroids: Anisaldehyde Sulphuric acid as regent and heat the plate at 105° C for 5 min. Shows, violet, blue, red, gray or green). Spraying is done in a TLC spray cabinet or in the fume hood. Change the bottle of the sprayer with up to 5ml of reagent. Place plate in a spray cabinet and spray plate until it is homogenously covered with the reagent. Dry plate with cold air and proceed with handling. Each developed plate is documented under 254 nm, 366nm and 520nm in densitometry TLC scanner 3.The CAMAG make HPTLC instrument were used for this analysis.

2.6 Statistical analysis:
Statistical comparison of the data was performed by one-way analysis of variance followed ‘t’ test using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. All solvent extracts were compared with standard drug against microorganisms, *P<0.05, ** P<0.01, *** P<0.001 were considered significant

3. RESULTS AND DISCUSSION

3.1 Phytochemical screening
Phytochemical screening is to isolate various constituents of the plants for assessing their biological activity or medicinal uses. The medicinal value of plants is due to the existence of exacting phytoconstituents that have a definite physiological role on the living system. Phytochemical screening is important to identify the secondary metabolites which are present in the leaf extracts for assessing their therapeutic activity. The medical value of this leaf extracts is due to the presence of particular secondary metabolites that have a definite physiological action on the living system. The secondary metabolites present in the methanol, ethyl acetate and aqueous extract were tabulated in table 1. The therapeutic value of leaf extracts is due to the presence of bioactive constituents. Flavonoids, tannins, phenols, terpenoids, carbohydrates, saponins, alkaloids, cardiac glycosides, proteins, sterols, tri terpenoids were present in methanol and ethyl acetate extract except aqueous extract. Fixed oils are absent in all extract. According to the Bipul Biswas [2], Psidium guajava methanol extract has absence of saponins. Balaji kaveti et al. [3] shows alkaloids, tannins and phenolic substances observed in alcohol and water
extract of Piper betle. In water extract, saponins and glycosides were present in the alcoholic extract. But in our combination of two leaf extracts it is present may be due to have synergistic effect. Pharmacological effects of betel chewing include abundant flow of saliva, temporary dulled of taste perception, stimulation of muscular and mental efficiency [4]. Phenolic compounds widely distributed in all plants have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic. Due to presence of phenolic compounds these might play role in the prevention of several chronic diseases such as cardiovascular disease, cancer, diabetes, bacterial and parasitic infections [5]. Flavonoids can also inhibit the activity of many enzymes such as xanthine oxidase, peroxidase and nitric oxide synthase, which are supposed to be involved in free radical generation, thereby resulting in decreased oxidative damage of macromolecules [6]. Tannins act as astringent, antioxidants, free radical scavengers; promote healing of wounds and effective in peptic ulcers while presence of reducing sugars in these plants has reductive properties [7]. Saponins which are glycosides have been found to have inhibitory effects on gram-positive organism, S. aureus. Steroids are frequently used signaling molecules biologically and decrease fluidity of membranes [8].

Table 1. Phytochemical screening of various solvent using PGBP leaf extract

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Methanol</th>
<th>Aqueous</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Phenols</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Saponins</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Cardiac Glycosides</td>
<td>Present</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Proteins</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Sterols</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Tri terpenoids</td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>Fixed oils</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
</tbody>
</table>

3.2 Antimicrobial activity

The efficacy of this plant extract (in combination of PGBP) was tested against Micrococcus sp., Streptococcus aerogenes, Neisseria sp., Vibrio sp., Shigella sp., Pseudomonas sp., E.coli, Bacillus subtilis, Bacillus sp., and Shigella sp. The methanol and aqueous extract shows very significant inhibition on growth of the tested microorganisms. Ethyl acetate extracts were found to be very least effective against most of the tested organisms. The methanolic extract was considerably more effective than aqueous extract in inhibiting the investigated microbial strains. Our results are supported by Nair R and Sumitra Chanda [9]. According to Bipul Biswas et al.[2], Only Gram-positive bacteria, Bacillus cereus and Staphylococcus aureus were susceptible to the methanol and ethanol extracts of Psidium guaiava, while neither of the Gram-negative bacterium showed any inhibition. But in our combined leaf extract, methanol and aqueous extract shows very significant inhibition against gram negative bacterium (E.coli). Nascimento et al.[10] conducted a study which supports the finding of the present study in which the guava extract was able to have inhibitory effects against Staphylococcus and Bacillus and no effect on the Escherichia and Salmonella. In methanol extract, maximum inhibition was observed on Shigella flexneri (25±0.00mm) and it is extremely statistically significant against standard at p<0.001. Minimum inhibition was observed on Bacillus sp.(21±1.0mm). In aqueous extract, maximum inhibition was observed on Shigella sp. (24.33±1.15mm) and Bacillus subtilis (24.33±2.08), minimum inhibition was seen in Micrococcus luteus (20.33±1.53mm). Among three extract, ethyl acetate extract shows least inhibition against Neisseria sp.(18±1mm). PGPS extracts were effective against both Gram positive and Gram negative bacteria. Antimicrobial activity of this leaf extracts were mainly due to the presence of secondary metabolites such as phenols, alkaloids, glycosides flavanoids and tannins. Antimicrobial activity in ethanoll, petroleum ether and chloroform extract of piper betel shows inhibition of Bacillus subtilis with the diameter range of 20-25mm [11] which merely similar to our result the inhibition of Bacillus range from 18-24mm. Ethyl acetate extract of Piper betel var. Bangladesh showed the maximum zone of inhibition (30mm) against Staphylococcus aureus followed by zone of inhibition (33mm) against Pseudomonas aeruginosa and the ethanolic, methanolic, ethyl acetate and aqueous extracts of Piper betel var. Jaleswar posses good antibacterial against Staphylococcus aureus and E.coli [12].
Table 2: Antimicrobial activity in various solvent extract in combination of PGPB

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Methanol Test</th>
<th>Methanol standard</th>
<th>Aqueous Test</th>
<th>Aqueous standard</th>
<th>Ethyl acetate Test</th>
<th>Ethyl acetate standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micrococcus luteus</td>
<td>21.67±3.06a</td>
<td>27.33±1.15</td>
<td>20.33±1.53b</td>
<td>27.67±1.53</td>
<td>19.3±1.76</td>
<td>22.3±0.67</td>
</tr>
<tr>
<td>Streptococcus aerogens</td>
<td>21.33±0.58b</td>
<td>26.67±1.15</td>
<td>22.67±1.15a</td>
<td>28±1.73</td>
<td>22±2.08</td>
<td>22.7±0.33</td>
</tr>
<tr>
<td>Neisseria sp.</td>
<td>23.67±2.52c</td>
<td>27.67±0.58</td>
<td>22.67±1.15b</td>
<td>27.67±0.58</td>
<td>18±1a</td>
<td>23±0</td>
</tr>
<tr>
<td>Vibrio sp.</td>
<td>23.33±3.21a,</td>
<td>29±0.0</td>
<td>23.33±2.08a</td>
<td>28±0.00</td>
<td>19.3±2.38</td>
<td>21.3±0.88</td>
</tr>
<tr>
<td>Shigella sp.</td>
<td>25±0.00d</td>
<td>28.33±0.58</td>
<td>24.33±1.15a</td>
<td>27±0.00</td>
<td>19±2</td>
<td>23.3±0.88</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>24±1.73b</td>
<td>26±5.29</td>
<td>23.67±1.53b</td>
<td>28±0.00</td>
<td>20±2.31</td>
<td>23.3±0.88</td>
</tr>
<tr>
<td>E.coli</td>
<td>23.33±1.53a</td>
<td>27.67±0.58</td>
<td>24±1b</td>
<td>28±0.00</td>
<td>21±1</td>
<td>23.8±0.88</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>21±1.00a</td>
<td>27.33±2.52</td>
<td>24.33±2.08a</td>
<td>28.67±0.58</td>
<td>18.33±0.88a</td>
<td>23.3±1.2</td>
</tr>
</tbody>
</table>

Mean±SD, n=3, a statistically significant against standard at<0.05; b very statistically significant at p<0.05 against standard; c significant at <0.1 against standard; d extremely significant at p<0.001 against standard; standard – Amoxicillin.

M- Methanol extract of PGPB; S-Standard (Amoxicillin); C- Control (Methanol)
Mahfuzul Hoque et al.[13] found no antibacterial activity of ethanolic extracts of guava against E. coli and S. enteritidis; however Vieira et al. 2006 found guava sprout extracts were effective against inhibiting E. coli. Sanches et al.[14] found that the aqueous extract of guava was effective against Staphylococcus and Bacillus. The methanolic extracts of guava reported by Lin et al.[15] showed significant inhibitory activity against the growth of two isolates of Salmonella and enteropathogenic E. coli. These results support our findings. The inhibition was more in combination of leaf extracts than compared to the individual leaf extracts. The antimicrobial activity of leaves of PGPB shows the presence of broad spectrum of antimicrobial compounds which act against gram positive and gram negative bacteria especially in methanol, aqueous and ethyl acetate. Methanol and aqueous extract of PGPB combination shows most significant zone of inhibition against many microorganisms compared to ethyl acetate.
extract. Further studies only carried out in methanol and aqueous extract.

3.3 High Performance Liquid Chromatography (HPTLC):

Herbal medicines are composed of many constituents and are therefore very capable of variation. As a result, it’s important very critical to attain reliable chromatographic fingerprints that constitute pharmacologically active and chemically feature additives of the herbal medicine. HPTLC fingerprinting profile may be very essential parameter of herbal drug standardization for the right identification of medicinal flowers. Those techniques have been also employed to analyze commercial samples to illustrate their utility in qualitative (fingerprint) and quantitative determination, demonstrating their feasibility in the excellent manages of phytoconstituents from stated herbal pills and formulations. This will help induced to come out uniform standard products, which will restore faith of product and alternative herbal medicine therapy. The TLC visualization without derivation was measured at 254nm and 366nm (Figure 2A,B). The TLC Visualization of chromatograms using Libermann burchard reagent (Fig. 2C) and Aluminum chloride (Fig.2D) showed different colours i.e. blue for tri terpenes and orange colour for flavanoids. Mean while, the appearance of numerous phytochemical constituents in the methanol and aqueous leaf extract PGPB in combination. Although, the TLC analysis is the simplest and cheapest method in getting fraction and separation in short time [16], it is proven that a suitable solvent system is necessary to obtain the best separation. We agreed their statement. A good separation obtained from the methanol leaf extract PGPB in combination compare to aqueous extract was resulted from solvent mixture used with its various polarities in different ratio.

![Figure 2 A,B,C,D- Visualization of methanol and aqueous extract of PGPB in combination (Mobile phase-Butanol: Acetic acid: Water (4:1:1))](image)

The results from HPTLC finger print (Figure 3) scanned and chromatogram shows the peak display (figure 5) at wavelength 254 nm and 366nm for methanolic extract of PGPB combination of leaf extract. HPTLC of methanolic extract have nine polyvalent phytoconstituents with their Rf value 0.12, 0.16, 0. 19, 0.23, 0.62, 0.75, 0.86, 0.91, 0.98 at lower and 0.14, 0.21, 0.27, 0.39, 0.53, 0.69, 0.82, 0.88, 0.97 at higher wavelength of solvent butanol, acetic acid ,water (4:1:1) and in derivatization @520nm, depending on the spraying reagent peak Rf value may varied. Methanolic extract has 14 Rf value such as 0.14, 0.18, 0.28, 0.32, 0.38, 0.39, 0.65, 0.68, 0.72, 0.76, 0.82, 0.86, 0.90, 0.98 when using derivatization for the identification of triterpenes. Libermann Burchard reagent shows more peaks and the presence of high quantities of phytoconstituents.component number 14 at 1.10 at Rf value showed maximum concentration. Our results coincide with Rashmi Tambe et al., 2014 [17]. They reported as in methanolic extract of Psidium guavaja, shows three polyvalent phytoconstituents with their Rf value 0.95, 1.11, 1.41, Component number 3 at 1.41at Rf value showed maximum concentration at 220nm. But we have 10 Rf value at 254nm because due to the combination of Piper betle with Psidium guavaja. When we use combination of plant products, it will enhance the therapeutic activity. But compare to aqueous extract only five Rf value 0.13,0.55,0.62,0.69,0.98 at wavelength @254nm and Rf value at only 4 peaks 0.14,0.31,0.36,0.62 and in derivatization at 520nm using Libermann reagent 12 Rf values 0.06 ,0.08, 0.13, 0.17, 0.46, 0.71, 0.74, 0.8, 0.92, 0.95, 0.97, 0.98 and using aluminum chloride spray six peaks were seen. The Rf values are 0.11, 0.14, 0.19, ...
0.67, 0.73, 0.98. According to Lavanya and Brahmapraksh [18], Rf values on the mobile phases uses, where compound that possessed higher Rf valued denoted low polarity while compound with lower Rf value indicated high polarity. In addition, Shalfiei stated that the different visualization techniques either viewed (long and Short UV) or normal light as well as assisted by chemicals, also gave different range of Rf values. Thus, the use of appropriate visualization aid needs to be consistent. The presence of terpenoids and flavanoids compounds might play role in the prevention of several chronic diseases such as Rheumatoid arthritis, cardiovascular disease, cancer, diabetes, bacterial and parasitic infections. Flavonoids can also inhibit the activity of many enzymes such as xanthine oxidase, peroxidase and nitric oxide synthase, which are supposed to be involved in free radical generation, thereby resulting in decreased oxidative damage of macromolecules [6]. When flavanoids inhibit xanthine oxidase, uric acid production will be blocked, in turn decrease the adverse effects of gouty arthritis by preventing the accumulation of uric acid in joints. It will cause severe pain in both gouty and rheumatoid arthritis patient.

Figure 3: Fingerprinting of methanol and aqueous extract of PGPB in combination (Mobile Phase-Butanol: Acetic Acid : Water(4:1:1))
Figure 5 Chromatogram: HPTLC of methanol and aqueous extract of PGPB in combination (Mobile phase-Butanol: Acetic acid : Water(4:1::1))
When they take these methanol extract is formulated as drug, it may decrease their pain. Figure 6 and 7 clearly shows that methanol extract has many compounds compare to aqueous extract in mobile phase of solvent system are Toluene, Ethyl Acetate and Methanol (7:2:1). The results from HPTLC finger print scanned at wavelength 254 nm and 366nm for methanol extract of PGPB leaf powder in combination showed the presence of total ten components in both wavelength with their Rf value 0.04, 0.10, 0.18, 0.22, 0.26, 0.26, 0.41, 0.51, 0.69, 0.79 ,0.90 and 0.06, 0.09, 0.17, 0.20, 0.27, 0.35, 0.42, 0.57, 0.63, 0.70, 0.79, 0.90, 0.94 respectively. Component number 10 at 0.90 and 0.94 Rf value showed maximum concentration. Similar results were seen methanol leaf extract of Psidium guavaja reported by Rashmi Tambe [17]. In his study, he reported that methanol extract of Psidium guavaja has 3 Rf value at 220nm and 4 Rf value at 450nm

![Figure 6: Chromatogram: HPTLC of methanol and aqueous extract of PGPB in combination (mobile phase -toluene: ethyl acetate: methanol (7:2:1))](image)

In our study, we had seen ten Rf value in lower wavelength and higher wavelength indicates combination of two leaves increase the phytoconstituents. It may increase the possibility of anti arthritic effect. In aqueous extract, 254nm wavelength has 3 Rf value and 466nm has 1 Rf value. By comparing the methanol and aqueous extract, methanol has more constituents; it will become effective drug for arthritides. In this mobile phase, we can identify steroids by spraying Anisaldehyde Sulphuric acid reagent. In visualization plate (Fig 7 C) clearly seen methanol extract has more bands than aqueous extract. HPTLC chromatogram of methanolic and aqueous extract results showed that there are many compounds in PGPB in
combination. From the HPTLC studies, it has been observed that methanol & aqueous extracts contain no longer a single compound however a aggregate of compounds and so it is established that the pharmacological activity shown by using them are because of the cumulative effect of all of the compounds in composite. It may be concluded that HPTLC fingerprint evaluation of leaf powder extract of Psidium guajava and Piper betle in aggregate can be used as a diagnostic tool for the precise identity of the plant and it is useful as a phytochemical marker and also an amazing estimator of genetic variability in plant populations.

**Figure 7** - visualization of methanol and aqueous extract of PGPB in combination (Mobile Phase; Toluene: Et. Acetate: Methanol (7:2:1))

4. CONCLUSION:
The present study showed that PGPB has more antibacterial activity against many human pathogens. The methanol extract has more antimicrobial activity compared to aqueous and ethyl acetate extract. We conclude that results of the present study contribute towards validating the traditional use of PGPB methanol extract formulation in the treatment of infection.

5 ACKNOWLEDGEMENT:
We would like to express our sense of gratitude to the Management of V.V.Vanniaperumal College for women, Virudhunagar for their financial assistance for this project and grant permission to do this work in our college and also thank “DST-FIST” of V.V.Vanniaperumal College for Women, Virudhunagar for utilizing the Internet Facilities. We immensely thank the N.Ramavarier Ayurveda foundation, AVN group, Madurai, Tamilnadu, India for the HPTLC analysis.

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