Piper betle L.: A review on its ethnobotany, phytochemistry, pharmacological profile and profiling by new hyphenated technique DART-MS (Direct Analysis in Real Time Mass Spectrometry).

Kushagra Nagori*, Mukesh Kumar Singh, Amit Alexander, Tekeshwar Kumar, Dhansay Dewangan, Hemant Badwaik, D.K. Tripathi
Rangta College of Pharmaceutical Sciences and Research, Bilahi 940006, Chhattisgarh, India.
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ABSTRACT

The Piper betle plant is an evergreen and perennial creeper which is used in several traditional medicines to cure various diseases. This plant has been known to possess antioxidant, antifungal, antileucocytic, antispasmodic, anthelminthic, anti-inflammatory, anti-rheumatic and antimicrobial activity. A wide range of chemical compounds including chavibetol, allyl pyrocatechol, eugenol, quercetin, caryophyllene, safrole, hydroxychavicol, α-pinene, myrcene, chavicol, Germacrene-D, α-terpineol, β-pinene, camphene etc have been isolated from this plant. The present review summarizes the information concerning the botany, ethnopharmacology, phytochemistry, biological activity and use of hyphenated analytical technique like DART-MS (Direct Analysis in Real Time Mass Spectrometry) and other techniques for characterizing various compounds from P. betle plant.

Key words: P. betle, DART-MS, creeper, antioxidant, chavibetol.

INTRODUCTION

Occurrence, Botanical description and Ethnopharmacology

From the ancient time, man has been using plants as medicine. From a historical perspective, it has been evident that the fascination with plants is as old as mankind. Herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. The plant kingdom represents a rich source of organic components, many of which have been used for medicinal & other purposes. Herbal medicines remain the major source of health care for the world’s population. Currently, there is growing interest in plant based or herbal medicines even in the western world. In many respects, the mechanism of action of the herbal drugs differs from that of the synthetic drugs or pure compounds. In one of the study of the World Health Organization it is estimated that 80% of the population of developing countries relies on traditional plant based medicines for their health requirements.

Piper betle Linn. (Bette vine) is a tropical plant closely related to the common pepper and belongs to the family Piperaceae. It is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries and has a long history of over 2000 yrs. The stems are dichotomous, articulate, swollen and rooted at nodes 3mm in diameter, woody and with 2.5 to 4cm long internodes. Stem stout with pinkish-shiie all over node dilated and rooting. The leaves are simple, spiral and exstipulate. The petiole is 5mm long, chan- neled and pubescent. The blade is 10 x 6cm & 9.5 x 5cm, ovate to ovate – acuminate, the secondary nerves are in three pairs. The inflorescence is an axillary spike which is 5.5 cm long. The flowers are drapaceous, orange, and 3mm in diameter. The betel leaf is known as Paan (Assamese, Urdu, Hindi, Odia, Bengali) and Bhakhshiyapatra, bhujangalata, bhujangavali, divabbhishta, kalakasanda, nagavalli, nagavallika, nagini, partha, vatiha (Sanskrit). Some of the names in the regions in which it is consumed are: betel leaf vine (English), Vettilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache pan (Marathi), Veeleyada (Tamil), Vetrilai (Tamil), Tamalapaku (Telugu), Vidyache
Kushagra Nagori et al. / Journal of Pharmacy Research 2011,4(9),2991-2997

PHYTOCHEMISTRY

The chemical constituents of betel essential oil consist of mainly terpenes and phenols. Leaves contain protein 3.1 %, carbohydrate 6.9 %, minerals 2.3 %, and tannins 2 %. Leaves contain bitter compounds that are about 0.7 to 2.6 %. The nutritional composition of fresh *Piper betle* leaf: water (85-90%), Protein (3-3.5%), Fat (0.4-1.0%), minerals (2.3-3.3%), Fibre (2.3%), Chlorophyll (0.01-0.25%), Carbohydrate (0.5-6.10%), Nicotinic acid (0.63-0.89mg/100g), Vitamin C(0.005-0.01%), Vitamin A (1.9-2.9 mg/100g), Thiamine (10-70 µg/100g), Riboflavin (1.9-30 µg/100g), Tannin (0.1-1.3%), Nitrogen (2.0-7.0%), Phosphorus (0.05-0.6%), Potassium (1.1-4.6%), Calcium (0.2-0.5%), Iron (0.005-0.007%), Iodine (3.4 µg/100g), essential oil (0.08-0.2%), Energy (44 kcal/100g). The *betel* leaves have strong pungent aromatic flavor and the characteristic flavor of betel is due to the betel phenols. More recent work with the leaves was found to contain starch, diastases, sugars (0.8 to 1.8 %) and an essential oil to an extent of 4.2%, the specific gravity varies from 0.938 to 1.057°. Phytochemical investigations on leaves revealed the presence of tannins and steroidal components. The terpenoids include 1,8-cineole, cadinene (Fig.1.4), camphene, carvophyllene, limonene, pinene, chavicol, allyl pyrocatechol, carvacrol (Fig.1.8), safrole, eugenol, and chavibetol are the major phenols found in *Piper betle*, there acetates are also commonly found. Eugenol was identified as the antifungal principle in the oil. Some of the phytoconstituents of the plant are summarized in the Table 2.

Table. 2. Phytoconstituents of *Piper betle*

<table>
<thead>
<tr>
<th>Active principle</th>
<th>Plant part used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chavicol</td>
<td>Leaves</td>
<td>57,22,54,56,59,60</td>
</tr>
<tr>
<td>Allyl-pyrocatechol</td>
<td>Leaves</td>
<td>58</td>
</tr>
<tr>
<td>Chavibetol-acetate</td>
<td>Leaves</td>
<td>61,62,57</td>
</tr>
<tr>
<td>Eugenol (Fig.1.1)</td>
<td>Leaves &amp; Flower</td>
<td>63,56,60</td>
</tr>
<tr>
<td>Alky-l-pyrocatechol</td>
<td>Leaves</td>
<td>65,66,64</td>
</tr>
<tr>
<td>Quercetin</td>
<td>Leaves</td>
<td>67,68</td>
</tr>
<tr>
<td>Luteolin, stearealdhyde</td>
<td>Leaves</td>
<td>68, 42</td>
</tr>
<tr>
<td>α-terpinol, β-pinen, camphene</td>
<td>Leaves</td>
<td>62</td>
</tr>
<tr>
<td>Caryophyllene (Fig.1.9)</td>
<td>Leaves</td>
<td>57,69,28</td>
</tr>
<tr>
<td>Safrole</td>
<td>Leaves</td>
<td>63,64,70,64</td>
</tr>
<tr>
<td>Hydroxychavicol</td>
<td>Leaves &amp; Flower</td>
<td>56,71,64</td>
</tr>
<tr>
<td>β-strokel</td>
<td>Leaves &amp; Roots</td>
<td>56,72,72</td>
</tr>
<tr>
<td>Diosgenin</td>
<td>Leaves</td>
<td>56</td>
</tr>
<tr>
<td>β-lactone, allyl catechol, eugenol methyl ether, cephradione A, dotticosterone, triacitone</td>
<td>Stem</td>
<td>73,72</td>
</tr>
<tr>
<td>Pipercornumine</td>
<td>Stem</td>
<td>73</td>
</tr>
<tr>
<td>4-allyl resorcol, humulene (Fig.1.17), stigmast-4-en-3-one, dehydroabietyl acetate A-II, 8-isopinocembrin, 8-β-acetyl isooric acid, α-sumaric acid</td>
<td>Roots</td>
<td>73</td>
</tr>
<tr>
<td>Linalool (Fig.1.10)</td>
<td>Leaves &amp; Rhizome</td>
<td>62,22</td>
</tr>
<tr>
<td>Alky-l diacetoxy benzen</td>
<td>Leaves</td>
<td>38</td>
</tr>
<tr>
<td>Gallic acid, procatechur acid, chlorogenic acid, caffeic acid, ferulic acid, ellagic acid</td>
<td>Whole plant</td>
<td>74</td>
</tr>
<tr>
<td>Isoeugenol-acetate</td>
<td>Whole plant</td>
<td>69,69</td>
</tr>
<tr>
<td>N-hexyl-2,4,6-decadienal, pipecadidi, pipecadine 4-allyl phenyl acetate, 4-allyl phenol, α- myrcetin, D-limonene, eucalyptol, camphor (Fig.1.15), allyl anisole, 4-allyl phenol, 8-isoo-safrole (Fig.1.18), thymol (Fig.1.18), 3-allyl-6-methoxyphenol, a-bergamotene, a-farnesene, a-muurolene, a-bergamotene, a-safrole, a-borneol, a-bisabolene, ledol, a-cadinol, isoledene</td>
<td>Whole plant</td>
<td>75</td>
</tr>
<tr>
<td>α-Isocopherol</td>
<td>Leaves</td>
<td>62</td>
</tr>
<tr>
<td>Eugenyl acetate, allyl pyrocatechol diacetate, allyl pyrocatechol monoacetate, terpen-4-ol, thiourene (Fig.1.6), camphene (Fig.1.15), sabine, 1,4 cienole, 8-isocineele, cis-sabinene hydrate, fenchone, terpinolene, 2-norborne, cis-limonene oxide, sabine (Fig.1.14), m-cymen-8-ol, cis-pipetol, α-decalyl, thymol, 2-undecanone, iso-sclareolide, α-sclareone, α-coparne (Fig.1.10), (E)-β-Damascenone, vanillain, α-selinene, coparne, germacrene A, n-eicosenate</td>
<td>Leaves</td>
<td>76</td>
</tr>
<tr>
<td>n-pine</td>
<td>Leaves</td>
<td>63,29</td>
</tr>
<tr>
<td>Chavicol (Fig.1.2)</td>
<td>Leaves</td>
<td>63</td>
</tr>
<tr>
<td>Myrcene (Fig.1.3)</td>
<td>Leaves</td>
<td>63</td>
</tr>
<tr>
<td>Isoeugenol</td>
<td>Leaves</td>
<td>63,64,62</td>
</tr>
<tr>
<td>β-βisopropyl palmitate, dodecanonic acid, septic acid, cepharadione-A</td>
<td>Leaves</td>
<td>72</td>
</tr>
<tr>
<td>Methyl eugenol, flavones</td>
<td>Leaves</td>
<td>64</td>
</tr>
<tr>
<td>Benzene acetic acid, hirdatecnic acid, octadecanonic acid, myristic acid,2,3-hex (hydroxy) propyl ester, 2 mono palmitin, limonone (Fig.1.7), octadecanonic acid, 2,3-bis(hydroxy) propyl ester</td>
<td>Leaves</td>
<td>71</td>
</tr>
<tr>
<td>Isoeugenyl-acetate</td>
<td>Leaves</td>
<td>69</td>
</tr>
<tr>
<td>(E)-6-ocisinele, terpinolene, allo-scinene, cymene (Fig.1.13)</td>
<td>Leaves</td>
<td>27,28</td>
</tr>
<tr>
<td>β-caryophyllene</td>
<td>Leaves</td>
<td>77,82,22</td>
</tr>
<tr>
<td>Germacrene-D (Fig.1.12)</td>
<td>Leaves</td>
<td>62</td>
</tr>
</tbody>
</table>

(1) Eugenol (C_{10}H_{18}O)_{2} (2) Chavicol (C_{16}H_{20}O)

(3) Myrcene (C_{10}H_{14}) (4) Cadinene (C_{15}H_{24})

(5) Camphene (C_{10}H_{16}) (6) Thujene (C_{10}H_{18})

(7) Limonene (C_{10}H_{16}) (8) Carvacrol (C_{10}H_{14}O)

(9) Caryophyllene (C_{15}H_{14}) (10) Linalool (C_{10}H_{18}O)

(11) α-copaene (C_{15}H_{24}) (12) Germacrene D (C_{15}H_{24})

(13) Cymene (C_{10}H_{14}) (14) Sabine (C_{10}H_{14})
fractionation to yield allylpyrocatechol (APC) as the major active principle in China in the prevention of oral malodor was examined by bioassay-guided treatment of halitosis. About 80, 28, and 73 concentrations of CHV and APC were significantly less in the sweet and betel genol, and peroxidation (LPO) of liposomes and rat brain homogenates as well than 4µg/ml in all the in vitro experiments. It could prevent Fe (II)-induced lipid higher peroxidation. Mysore varieties. Among the isolated compounds, APC showed the best results regarding that can be correlated with the total phenolic content and reducing powers revealed similar chemical profiles in all three varieties that were used in India and China in the prevention of oral malodor. Two varieties of Piper betle (green and red vein) showed strong activity against all the pathogens tested (Colletotrichum capsici, Fusarium pellitorum, Botryodiplodia theobromae, Alternaria alternata, Penicillium citrinum, Phomopsis caricae-papayae and Aspergillus niger) with inhibition diameters significantly (P < 0.01) bigger than 2-5 mg/ml prochloraz or 10 mg/ml clotrimazole. The minimum inhibitory concentrations of the ethanolic extracts of P. betle against these plant pathogens ranged between 0.01 mg/ml and 1 mg/ml.

**Antifungal activity**

An ethanolic extract of leaves of Piper betle (PB) was tested for its antifungal activity. A study indicated that the potential to reduce methylmercaptan and hydrogen sulfide was mainly due to the anti-microbial activity as established using dynamic in vitro models. The adhering ability of microorganisms to the tooth surface determines the developmental stage of dental plaque. With suitable environmental and nutrients, cariogenic plaque will subsequently develop. To minimize the development of cariogenic plaque, the adhering ability of the early settlers needs to be controlled. The aqueous extract of P. betle is used which reduced the adherence of early plaque bacteria to experimental pellicle. The extracts were observed to have the ability to make them less adherent. This could account for the significant reduction in the hydrophobic binding capacity of the bacteria when treated with the extracts.

**Antileishmanial activity**

An ethanolic extract of leaves of Piper betle (PB) was tested for its antileishmanial activity which was evidenced in both promastigote and amastigote, with IC50 values of 9.8 and 5.45 µg/ml, respectively; importantly, it was accompanied by a safety index of 12-fold. This leishmanicidal activity of PB was mediated via apoptosis as evidenced by morphological changes, loss of autophondial membrane potential, in situ labeling of DNA fragments by terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end labeling, and cell-cycle arrest at the sub-G0/G1 phase. It is anticipated that phenolics identified in PB such as APC and chavibetol could contribute towards the observed antileishmanial activity.

**Radioprotective activity**

The radioprotective activity of Piper betle ethanolic extract has been studied using rat liver mitochondria and pBR 322 plasmid DNA as two model in vitro systems. The extract effectively prevented γ-ray induced lipid peroxidation as assessed by measuring thiobarbituric acid reactive substrates, lipid hydroperoxide and conjugated diene. Likewise, it prevented radiation-induced DNA strand breaks in a concentration dependent manner. The radioprotective activity of PE could be attributed to its hydroxyl and superoxide radicals scavenging property along with its lymphoproliferative activity. The radical scavenging capacity of PE was primarily due to its constituent phenolics, which were isolated and identified as chavibetol and allyl pyrocatechol.

**Antileucerogenic activity**

Pretreatment of an ethanolic extract of leaf of Piper betle Linn at a dose of 200mg/kg body weight, orally administered to rats for ten consecutive days, was found to possess a significant protective action against gastric lesions induced by indomethacin. The extract pretreatment resulted in a significant increase in superoxide dismutase (SOD) and catalase (CAT) activity, increase in mucus, hexosamine and total thiol group content, but marked reduction in oxidatively damaged protein and peroxidised lipid levels as compared to untreated ulcerated control. The extract was also found to possess both superoxide and hydroxyl free radical scavenging activity. The present observation establishes the efficacy of the extract in prevention of experimentally induced peptic ulcer by indomethacin and antioxidant property appears to be predominantly responsible for such cytoprotective activity in the experimental model. Further investigation showed the protective activity of allyl pyrocatechol (APC), which is the major antioxidant constituent of P. betle against the indomethacin-induced stomach ulceration in the rat model. This was done by treating the rat model with APC (2mg/kg body wt per day) and misoprostol (1.43 µg/kg body wt per day) for 7 days and effectively heals the stomach ulceration as revealed from the ulcer index and histopathological studies. The hexosamine content of APC treated group was not different from the ulcerated group. The extract has shown to possess both antioxidant and anti-inflammatory activities in the stomach ulceration model, which has been studied and compared with that of misoprostol. It was found that the excellent healing activity of ethanolic extract of P. betle play a major role of mucin protection and regeneration in the healing of non-steroidal anti-inflammatory drugs mediated stomach ulceration.
Antiplatelet activity
ROS (reactive oxygen species) are crucial for platelet aggregation and IPB extract has been found to inhibit the arachidonic acid (AA) induced and collagen-induced platelet aggregation, with an IC50 of 207 and 335 μg/ml, respectively. The IPB extract also inhibited the AA-, collagen- (>100μg/ml of IPB), and thrombin (>250 μg/ml of IPB)-induced thromboxane B2 (TXB2) production, but the effect of thrombin-induced aggregation was not significant. These results indicated that aqueous components of IPB are potential ROS scavengers and may prevent the platelet aggregation possibly via scavenging ROS or inhibition of TXB2 production.

Immunomodulatory activity

Many of the disorders today are based on the imbalances of immunological processes. This necessitates the search for newer and safer immunomodulators. With this objective a study was conducted to explore the immunomodulatory activity of the methanolic extract of Piper betle L. The MPb consists of mixture of phenols, flavonoids, tannins and poly saccharides. Both in vitro as well as in vivo evaluation was carried out. The effects of MPb on lymphocyte proliferation, interferon-γ receptors and the production of nitric oxide were measured in vitro. Further, the extract at different dose levels was studied in vivo for the humoral and cellular immune responses on mice immunized with sheep red blood cells, the result showed that P. betle significantly suppressed phytohaemaglutinin stimulated peripheral blood lymphocyte proliferation in a dose-dependent manner. The decrease in antibody titre and increased suppression of inflammation suggests possible immunosuppressive effect of extract on cellular and humoral response in mice. The study indicated the potential of MPb as a novel candidate for immunosuppressive activity. The same could be further evaluated for its anticancer activity or as a potential candidate in the treatment of autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus or emphysema.

Antimicrobial activity

The ethanolic and aqueous extracts showed strong activity against the yeasts: C. albicans, and M. pachydermatis. The crude essential oil exhibited a broad-spectrum strong antimicrobial activity against all test organisms. The strongest activity was observed against C. albicans, followed by S. aureus and M. pachydermatis. Further an investigation reported the antimicrobial activity of crude aqueous extract of P. betle on Streptococcus mutans. Transmission electron microscopy (TEM) was used to determine the effect of the extract on the ultrastructure of S. mutans. The study showed cells with nuclear material coagulated in to thick electron dense filaments and destruction of the plasma cell membrane and inner cell wall. The effect is more significant with the higher concentration of the extract. The effect of crude extract on the ultrastructure of S. mutans observed in this study could be due to the fatty acids and hydroxyl fatty acid ester components present. The hydrophobic parts of the compound may enable them to partition the lipids of the bacterial cell membrane, thereby disturbing the structures and rendering them more permeable. When the membrane is more permeable, other components present in the extract could make its way in to the bacterium and coagulate the nucleoid while maintaining the cell intact. Analysis of the effect on the acid producing properties was analysed by pH drop assay. The extract was found to suppress the acid producing properties of the bacteria. The anti-giardial activity of P. betle was checked against 15 clinically important bacterial strains, showing moderate activity against B. cereus. Here cefotaxime (100µg/disc) was used as a standard. From results it can be said that P. betle can be explored further as they are able to resist bacterial growth up to some extent.

Mosquito larvicidal and Tyrosinase inhibition activity

While mosquito larvicidal activity was carried out with the essential oil and methanolic and aqueous extracts of P. betle. The essential oil exhibited the larvicidal activity significantly with 2 h and 24 h, LD50 value of 86 and 48 ppm, respectively. The methanolic extract of P. betle showed larvicidal activity with 2 h and 24 h with LD50 value of 153 and 125 ppm, respectively, whereas the aqueous extract showed slight activity. The study suggested that the essential oil and methanolic extract of P. betle is a potential natural mosquito larvicide. Inhibition of mushroom tyrosinase by the essential oil, methanolic and aqueous extracts of P. betle was evaluated. The essential oil exhibited concentration-dependent inhibition of mushroom tyrosinase, giving an IC50 value of 126 ppm. The essential oil was fractionated and two fractions 1 and 2 showed a strong inhibition of mushroom tyrosinase activity concentration-dependently, at an IC50 value of 115 ppm. The presence of the hydroxyl group at the 4 position of the aromatic ring (4-allylphenol, eugenol) in the essential oil, may play an important role in the inhibition of tyrosinase.

Antiinflammatory activity

The study reported that the crude leaf powder suspension of P. betle was evaluated for acute and chronic anti-inflammatory study at a dose of 300 mg/kg. Diclofenac sodium was used as the standard drug. Carrageenan and dextran models were studied for acute inflammatory study while cotton pellet induced granuloma was used for chronic inflammation study. ANOVA followed by Dunnett’s test were employed for statistical analysis. The study indicates that the P. betle exhibit significant anti-inflammatory activity.

Anti-amoebic & anti-giardial activity

Anti-amoebic activity of P. betle was studied with chloroform extract of P. betle leaf which was commonly used by patients in southern Thailand. It was screened at a concentration of 1,000 μg/ml against Entamoeba histolytica strain HTH-56: MUTM and strain HM1:IMSS growing in vitro. The extract was incubated with 2×105E. histolytica trophozoites/ml of medium at 37°C under anaerobic conditions for 24 h. The cultures were examined with an inverted microscope and scored (1-4) according to the appearance and numbers of the trophozoites the extract caused inhibition and retested using appropriate concentrations but with concentrations that ranged from 3.125 to 1,000 μg/ml/ml using E.histolytica strain HM1:IMSS, and the IC50 values for each extract was calculated. The chloroform extract of P. betle with IC50 value of 55.2 μg/ml was classified as active i.e. with an IC50 of less than 100 μg/ml. The IC50 was calculated against a standard drug metronidazole with an IC50 of 1.1 μg/ml for P. betle leaf which was commonly used as self medication by AIDS patients in southern Thailand. The standard drug metronidazole and plant extract was incubated with 2 x 10^6 trophozoites of Giardia intestinalis per milliliter of growth medium in 96-well tissue culture plates under anaerobic conditions for 24 h. The chloroform extract of P. betle was shown to be active i.e. with an IC50 of 100 < μg/ml the results shows that the MIC & IC50 values of P. betle leaf have potential use against infection caused by G. intestinalis.

ANALYTICAL TECHNIQUES USED IN IDENTIFICATION OF CHEMICAL CONSTITUENTS OF P. BETLE

Proflifer of Piper betle Linn. by Direct Analysis in Real Time Mass Spectrometric Technique.

Identification and quantitation of bioactive components from raw materials and processed products has been routinely performed by HPLC-PAD, HPLC- MS, GC-MS, etc. However, with the rapid advancements in mass spectrometry the need for more efficient and prompt analytical techniques in order to cope with the ever increasing number of compounds present in plant materials has been emerging. One such new technique is Direct Analysis in Real Time Mass Spectrometry (DART-MS). The new technique referred to as Direct Analysis in Real Time has been coupled to the AcquityTOF-LC atmospheric pressure ionization mass spectrometer to permit high-resolution, exact mass measurements of gases, liquids, and solids. Using a helium plasma DART ionizes atmospheric water and generates water clusters which in turn ionize the sample held in the gas stream. The resulting spectra are relatively clean and simple. As DART ion source can ionize molecules directly from the surface plant products, which can be analyzed directly without sample preparation. The eight varieties of Piper betle leaves were analysed by DART-MS named Bangla, Deswari, Deshi, Jaleshar green, Jaleshar white, Kalkatiya, Mahoba and Saufa. The results were shown in the Table 3.

Table 3. Exact mass data from the DART mass spectra of Piper betle leaves

<table>
<thead>
<tr>
<th>Molecular weight</th>
<th>Measured mass</th>
<th>Calculated mass</th>
<th>Molecular mass</th>
<th>Error (ppm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>134</td>
<td>135.0828</td>
<td>135.08699</td>
<td>CH3O</td>
<td>0.29</td>
<td>Charcoal</td>
</tr>
<tr>
<td>150</td>
<td>151.07640</td>
<td>151.07950</td>
<td>CH3O</td>
<td>0.50</td>
<td>Allylopyrocatechol</td>
</tr>
<tr>
<td>165</td>
<td>165.0924</td>
<td>165.09155</td>
<td>CH3O</td>
<td>0.87</td>
<td>Charcoal</td>
</tr>
<tr>
<td>165</td>
<td>166.08868</td>
<td>166.08446</td>
<td>CH3O</td>
<td>0.84</td>
<td>Phenyl salicylate</td>
</tr>
<tr>
<td>174</td>
<td>175.07765</td>
<td>175.07950</td>
<td>CH3O</td>
<td>1.74</td>
<td>Unknown</td>
</tr>
<tr>
<td>177</td>
<td>177.09119</td>
<td>177.09109</td>
<td>CH3O</td>
<td>-0.46</td>
<td>Chavicol acetate</td>
</tr>
<tr>
<td>192</td>
<td>193.08772</td>
<td>193.08647</td>
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<td>1.25</td>
<td>Allylopyrocatechol</td>
</tr>
<tr>
<td>206</td>
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<td>207.10122</td>
<td>CH3O</td>
<td>-0.72</td>
<td>Charcoal acetate</td>
</tr>
<tr>
<td>251</td>
<td>252.12287</td>
<td>252.12358</td>
<td>CH3O</td>
<td>-0.71</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Kushagra Nagori et al. / Journal of Pharmacy Research 2011,4(9),2991-2997

Journal of Pharmacy Research Vol.4 Issue 9 September 2011 2991-2997
The mass spectrometer used was a JMS-100 TLC (AccuToF) atmospheric pressure ionization time-of-flight mass spectrometer (Jeol, Tokyo, Japan) fitted with a DART ion source. The mass spectrometer was operated in positive-ion mode with a resolving power of 6000 (full-width at half-maximum). The orifice 1 potential was set to 28 V, resulting in minimal fragmentation. The ring lens and orifice 2 potentials were set to 13 and 5 V, respectively. Orifice 1 was set to a temperature of 100°C. The RF ion guide potential was 300 V. The DART ion source was operated with helium gas flowing at approximately 4.0 L/mn. The gas heater was set to 300°C. The potential on the discharge needle electrode of the DART source was set to 300 V; electrode 1was 100 V and the grid was at 250 V. Freshly cut pieces of betel leaf were positioned in the gap between the DART source and mass spectrometer for measurements. Data acquisition was from m/z 10 to 1050. Exact mass calibration was accomplished by including a mass spectrum of neat polyethyleneglycol (PEG) glycol (1:1 mixture PEG 200 and PEG 600) in the data file. m/z 244.0098 was used for calibration. The mass accuracy was accurate to within 0.002 u. Using the Mass Center software, the elemental composition was obtained from the DART-MS spectra.

APPLICATION OF DART – MS

DART has been interfaced to mass spectrometry for the analysis of counterfeit bioanalytical samples such as bioactive compounds, pharmaceuticals, and medical products. The mass spectrometer used was a JMS-100 TLC (AccuToF) atmospheric pressure ionization time-of-flight mass spectrometer (Jeol, Tokyo, Japan) fitted with a DART ion source. The mass spectrometer was operated in positive-ion mode with a resolving power of 6000 (full-width at half-maximum). The orifice 1 potential was set to 28 V, resulting in minimal fragmentation. The ring lens and orifice 2 potentials were set to 13 and 5 V, respectively. Orifice 1 was set to a temperature of 100°C. The RF ion guide potential was 300 V. The DART ion source was operated with helium gas flowing at approximately 4.0 L/mn. The gas heater was set to 300°C. The potential on the discharge needle electrode of the DART source was set to 300 V; electrode 1was 100 V and the grid was at 250 V. Freshly cut pieces of betel leaf were positioned in the gap between the DART source and mass spectrometer for measurements. Data acquisition was from m/z 10 to 1050. Exact mass calibration was accomplished by including a mass spectrum of neat polyethylene glycol (PEG) glycol (1:1 mixture PEG 200 and PEG 600) in the data file. m/z 244.0098 was used for calibration. The mass accuracy was accurate to within 0.002 u. Using the Mass Center software, the elemental composition was obtained from the DART-MS spectra.

CONCLUSION

The scientific research on P. betle suggests a huge biological potential of this plant. It is strongly believed that detailed information as presented in this review on the phytochemical, analytical techniques and various biological properties of the extracts of the plant can provide detailed evidence for the use of the plant in different medicinal applications. The phytochemical variations and efficacy of the medicinal values of P. betle depend on geographical locations and seasons. Betel quid are very commonly used by local people of India. Betel quid chewing is dependent on geographical locations and could be further exploited in the future as a source of useful phytochemicals.

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Kushagra Nagori et al. / Journal of Pharmacy Research 2011, 4(9), 2991-2997

Kushagra Nagori et al. / Journal of Pharmacy Research 2011, 4(9), 2991-2997

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