Screening of ethnomedicinal plants of diverse culture for antiviral potentials

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Since time immemorial Ethnomedicinal plants have been used for diverse ailments including infectious diseases. There is an increasing need for new anti-infective molecules, particularly from the plants used in ethnomedicinal practices, as the treatment of infectious diseases with the antimicrobial drugs frequently develops drug-resistance microbes. Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) causes a variety of diseases including herpes labialis, keratoconjunctivitis, encephalitis, herpes genitalis, and the lifelong latent infections in sensory nerve ganglia. Till date there is no effective anti-HSV vaccine, and the available drugs used against HSV infections have limited efficacy with frequent development of drug-resistant viruses. Here we have evaluated the anti-HSV potential of nine selected ethnomedicinal plant extracts of different families, traditionally used by diverse communities against skin, intestinal and sexual ailments, using wild-type and clinical isolates of HSV-1. The cytotoxicity of the extracts was determined on Vero cell by MTT assay; while the antiviral activity was screened by cytopathic effect reduction, MTT assay and plaque reduction assay. Interestingly the extracts of \textit{Dillenia indica}, \textit{Odina wodiera} and \textit{Moringa oleifera} exhibited significant antiviral activity against HSV-1 at non-cytotoxic concentrations; while the extracts of \textit{Morus alba} and \textit{Butea monosperma} showed antiviral activity at higher concentrations.

\textbf{Keywords:} Herpes Simplex virus, Antiviral efficacy, Plaque reduction assay, MTT assay

\textbf{IPC Int. Cl.}\textsuperscript{5}: A61P, A61K 36/00, A01D 4/26, A01D 4/36, A01D 4/40, A01D 10/00

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are enveloped, double stranded DNA virus of the family \textit{Herpesviridae} of subfamily \textit{Alpha-herpesviridae}, and are responsible for a number of diseases including herpetic labiales, ocular herpes, encephalitis, and herpes genitalis in targeted epithelial cells\textsuperscript{1}. Following host cell entry, HSV establish primary infection at the site of entry and then transported to the sensory ganglia for life-long latency with periodic reactivation to cause recurrent infections back to the entry site\textsuperscript{2}. Although HSV-1 is predominantly associated with oral and HSV-2 with genital infections, both can infect at either site. Since 1970, HSV infections were treated with the nucleoside analogues acyclovir and related pro-drugs valaciclovir, famciclovir, and penciclovir having poor oral bioavailability due to high polarity and low intestinal permeability, and are unable to eliminate the virus from the host\textsuperscript{3}. Moreover, the extensive and long-term use of these agents frequently develops drug-resistant viruses\textsuperscript{1, 2}, and may incorporate into the host DNA. Furthermore, there is no effective vaccine against HSV infections or its recurrences\textsuperscript{4}, and the present treatment can only reduce the virus number, severity and transmission\textsuperscript{3}.

Throughout the age’s mankind have relied on nature for healing by using poultices and infusions of local plants with mixed results; often it cures or relief symptom but sometimes poisonings occurred. The modern medicine has gradually developed over the years by observational and scientific efforts from traditional medicines, and even today the ancient wisdom of ethnomedicines is an important source of drug development. Ethnobotanical literature of diverse culture described the usage of folk-remedies including the plant extracts, infusions and powders for the management of diverse diseases including the viral diseases\textsuperscript{5}. However, there is an increasing need for new compounds with antiviral activity as the treatment of viral infections with the available antivirals are often unsatisfactory with the problem of resistance, viral latency and conflicting efficacy in recurrent infections\textsuperscript{6}. Interestingly, traditional medicines, like \textit{Ayurvedic}, Traditional Chinese...
Medicine (TCM), Chakma medicines, etc., were believed to be a good source for potential drug development. A wide variety of active phytochemicals including the alkaloids, coumarins, flavonoids, lignans, limonoids, organosulfur, peptides, polyphenolics, saponins, terpenoids, etc., are reported to have therapeutic applications against genetically and functionally diverse viruses, due to their broad range of bioactivities. Many of these compounds have antioxidant, free radical scavenging, and antiviral activities by inhibiting viral entry, replication, gene synthesis, or assembly. Ethnopharmacology provides an alternative approach for the drug discovery, as many ethnomedicinal plants used in traditional medicines have significant pharmacological activities by inhibiting viral entry, replication, gene synthesis, or assembly. Ethnopharmacology provides an alternative approach for the drug discovery, as many ethnomedicinal plants used in traditional medicines have significant pharmacological activities. A detailed investigation on the efficacy and safety of such plants, used in local healthcare, may help in the development of invaluable herbal lead.

Thus, we have selected nine ethnomedicinal plants, used for diverse ailment by different communities of India, to evaluate their efficacy against in vitro HSV-1 infection. These plants were traditionally used against skin, intestinal and sexual ailments by different tribal communities of Eastern and North Eastern India (Table 1). Moreover, it has been reported that these plants, for example *Dillenia indica* contain bioactive polyphenolics, flavonoids, terpenoids, and arabinogalactan; while *Moringa oleifera* contains nitriles, isothiocyanate and thiocarbanates, which may have clinical significance for the management of viral infections, including HSV-1. Hence, the present study was undertaken to evaluate the antiviral potential of nine selected plants and their Ethnopharmacological relevance with an aim to develop an herbal lead for the management of HSV-1 infection.

**Methodology**

**Plant materials**

The plant samples, selected on the basis of their traditional use, were collected from different locations of Eastern and North Eastern India, along with the relevant information of approximate dose and dosage form, frequency and duration of use from the respective users through a structured questioner, after obtaining their written and verbal consent, as and when required during our survey in 2012-2013 (Table 1). The samples were authenticated by a Taxonomist at the Botanical Survey of India, Howrah, and the Voucher specimens with the voucher number were deposited at the host institute.

**Extraction of the plant material**

The air dried and powdered plant materials were extracted with methanol (95%) for 72 hrs at room temperature. The extracts were then filtered and evaporated under vacuum at 40-45 °C using an Eyela Vacuum Evaporator to get the crude dried extract. All the extracts were stored at 4 °C until further use; and when required the extracts were dissolved in 0.1% dimethyl sulfoxide (DMSO) and diluted with the culture media, to obtain stock solution of 1 mg/ml.

**Viruses and the cell line**

African green monkey kidney cells (Vero cells, ATCC, Manassas, VA, USA) was grown and maintained in Eagle’s minimum essential medium (EMEM), supplemented with 5-10% fetal bovine serum (FBS, Invitrogen, USA). The standard strains of HSV-1F (ATCC 733) purchased from the ATCC (USA) along with a clinical strain of HSV-1 (VU-09), isolated from a patient, was used. After plaque purification, the viruses were grown and the virus stocks were stored at -80 °C for future use, and whenever required the virus stocks were grown on Vero cells to determine the titer(s) by plaque reduction assay for further study.

**Determination of cytotoxicity by MTT assay**

The MTT assay was used to monitor the cellular toxicity of the test extracts on Vero cell. Vero cell (1.0×10^5 cells/ml) monolayers cultured onto 96 well plates at 37 °C in 5% CO2 for 6 hrs was exposed to the different concentrations of the extracts at a final volume of 100 µl, in triplicate, using DMSO (0.1%) and Acyclovir (ACV) as a negative and positive control, respectively. The extract treated cells in each well were then incubated at 37 °C with 5% CO2 for 72 hrs and then added with the MTT reagent (10 µl). After 4 hrs incubation at 37 °C in 5% CO2, the formazan was solubilized by adding diluted HCl (0.04 N) in isopropanol, and the absorbance was read at 570 nm with a reference wavelength of 690 nm by an ELISA reader. The 50% cytotoxic concentration (CC_50) was calculated as:

\[
\text{CC}_{50} = \left( \frac{\text{sample absorbance-cell free sample blank}}{\text{mean media control absorbance}} \right) \times 100\%
\]

**Determination of antiviral activity by cytopathic effect reduction (CPE) and MTT assay**

The initial inhibitions of HSV-induced cell killing by the extract(s) was determined by CPE reduction...
<table>
<thead>
<tr>
<th>Plant name and Voucher No</th>
<th>Local name</th>
<th>Family</th>
<th>Parts used (Dosage, Frequency &amp; duration)</th>
<th>Traditional uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegle marmelos L. (Corr.) SNPS-JU/2014/2103</td>
<td>Bel</td>
<td>Rutaceae</td>
<td>Leaf paste (5-10 leaves) or juice used topically, or orally twice for a week. About 2-5 leaves sallow fried in cow ghee, and taken 2-3 times a day for 7 days to control dry cough of children. Fresh fruits burnt in cow dung fire, and then mixed with molasses to prevent acidity; while ripe fruits were taken twice daily to reduce constipation.</td>
<td>Leaf paste topically used for wound healing, urination, body odor, ear trouble, boils, dry cough, and abscess by local and tribal communities like Sabar, Lodha, Munda, Santal of West Bengal; Bodo and Kacharies of Assam. Other uses include as astringent, in eye problem, diarrhea, and dysentery; while root Juice for fever.</td>
</tr>
<tr>
<td>Butea monosperma (Lam.) Kuntze SNPS-JU/2014/1459</td>
<td>Palash</td>
<td>Fabaceae</td>
<td>Flowers (2-5) soaked overnight in a glass of water, filtered and taken in empty stomach. Leaf: One tablespoon of leaf decoction taken for 3 days after fifth day of menstruation for Conception. Seed powder mixed with goat milk as aphrodisiac and contraceptive.</td>
<td>Flowers are used in skin diseases, gout, high blood pressure, diarrhoea, leprosy, to reduce burning sensation; as astringent and contraceptive by Bhil, Movachi, Kokani, Warli, and Karkari. Leaf decoction and seed powder is used for conception by Santal, kandha, ganda and sabara tribes of Bengal and Orissa.</td>
</tr>
<tr>
<td>Dillenia indica L. SNPS-JU/2012/2102</td>
<td>Chalta</td>
<td>Dilleniaceae</td>
<td>Fruits and bark paste applied 2-3 times daily for 5-7 days to burst abscess, and relieve pain. Leaf paste used 3-4 times a day for 5 days in edema. Oil is applied twice daily for 10 days to reduce skin diseases.</td>
<td>Fruits are used to rupture abscess, relieve pain, and stimulate appetite; to prevent dandruff, fever, constipation, dysentery, and stomachache by Lepcha tribes of Dzongu valley, North Sikkim; and Apatami, Mongpa, Singpho, Tangsa tribes of Arunachal Pradesh. Oil is applied topically in skin diseases by Garo tribes.</td>
</tr>
<tr>
<td>Enhydra fluctuans Lour. SNPS-JU/2014/2101</td>
<td>Helencha</td>
<td>Asteraceae</td>
<td>Leaves (4-5) boiled in water (2 cups/100 ml) and taken in empty stomach for moths as blood purifier. Leaf juice (10 ml) mixed with juice of Centella asiatica and cucumber (2-5 ml) and taken twice for 3 weeks.</td>
<td>Leaf juice is externally applied in skin diseases, and to reduce prickly heat; and taken orally to purify blood by Jaintia and Garo tribes of Meghalaya. It is also used in hypertension and excess bile secretion.</td>
</tr>
<tr>
<td>Morus alba L. SNPS-JU/2013/1950</td>
<td>Tunt</td>
<td>Moraceae</td>
<td>Leaf paste of M. alba, Eupatorium odoratum, Euphorbia hirta, and Ficus benghalensis used to apply twice for 7 days to heal wounds.</td>
<td>Leaf used as antibacterial, diaphoretic, hypoglycemic and treating cuts and wound by Santal and Sabar tribes of West Bengal and Kuruma tribes of Kerala, India.</td>
</tr>
<tr>
<td>Moringa oleifera Lam. SNPS-JU/2012/1075</td>
<td>Sajina</td>
<td>Moringaceae</td>
<td>Flower: decoction of 2-3 flowers taken twice daily for 15 days to increase sperm</td>
<td>Used to prevent heat, treat indigestion, eye problems, cough, cold, inflammation and infections by the local and tribal communities.</td>
</tr>
</tbody>
</table>
production. While Pod pastes used twice daily for 7 days to reduce body heat, indigestion, and eye problems.

Bark (fresh) inhaled to clear nasal decongestion in acute cold.

**Odina wodier** Roxb.  *Kashmala* Anacardiaceae

Bark decoction used three times daily for 7 days for skin problems.

Leaf decoction: 5-10 ml daily for 7 days to treat cough.

Leaf paste applied three times daily to cuts, boils and scabies. Warmed leaves (2-5) help to stop nose bleed.

Leaf (2-4) heated in ghee and used for 5-7 days can reduce dry cough.

**Piper beetle** L. *Pan* Piperaceae

Leaf decoction: 5-10 ml daily for 7 days to treat cough.

Leaf paste applied three times daily to cuts, boils and scabies. Warmed leaves (2-5) help to stop nose bleed.

Leaf (2-4) heated in ghee and used for 5-7 days can reduce dry cough.

**Stereospermum suaveolens** Roxb. *Atkapali* Bignoniaceae

Paste of Root, Leaf, and Bark used twice daily for 7-10 days; or consume 2-3 tablets (5 gm paste) twice daily for 15 days.

Used for skin ailments, piles, inflammation, malaria, gonorrhea, and bronchitis by *Santal* and *Munda* of West Bengal; *Bodo* and *Miris* of Assam; *Garo* and *Khasi* of Meghalaya; *Kotas*, *Siddis*, *Badagas*, *Kurumbas*, and *Warlis* of Western Ghats.

Determination of antiviral activity by plaque reduction assay (PRA)

To further confirm the antiviral activity of the test extracts PRA was used in presence of ACV and DMSO (0.1%) as control. Serial dilutions of the test agents and ACV in EMEM was added to the virus-infected Vero cell monolayers (MOI: 1.0) and overlaid with overlay medium containing test agents, to enable the virus to spread via cell-to-cell route to form plaques. The plaques developed after 3 days of incubation were fixed with 4% paraformaldehyde and stained with methylene blue (0.03%) in 70% methanol. The effective concentration of the test extracts that inhibited the number of viral plaques by 50% (EC$_{50}$) was interpolated from the dose-response curves.

Results

Assessment of cytotoxicity and anti-HSV activity

The cytotoxicity & antiviral activity and bioactivity study of the test extracts, presented in Tables 2 & 3 revealed that three, out of 9 extracts tested, have significant antiviral activity against both the isolates of HSV-1 at a concentration that are non-cytotoxic to the Vero cells. The methanol extracts of *Dillenia indica*, *Moringa oleifera* and *Odina wodier* exhibited detectable antiviral activity against HSV-1F with EC$_{50}$ of 56.19, 74.8 and 22.4 μg/ml; while the human isolate VU-09 was inhibited (EC$_{50}$) at 61.2, 79.6 and 24.2 μg/ml, respectively. However, the extracts of *Enhydra fluctuans* and *Piper longum* showed anti-HSV activity at higher concentrations, but the other four extracts failed to produce detectable antiviral activity against the tested viral strains.

The results of CPE inhibition assay, followed by the MTT and plaque reduction assay were found to be comparable, and the extracts of five plants exhibited

Table 2—Assessment of cytotoxicity and antiviral activity of methanol extracts of 9 Plant against HSV-1

<table>
<thead>
<tr>
<th>Plants</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>HSV-1F (at MOI: 1.0)</th>
<th>VU-09 (HSV-1 isolate) at MOI of 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegle marmelos</td>
<td>32.4 ± 5.2</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Butea monosperma</td>
<td>329.2 ± 7.4</td>
<td>107.5 ± 3.9</td>
<td>3.06</td>
</tr>
<tr>
<td>Dillenia indica</td>
<td>427.4 ± 8.4</td>
<td>56.19 ± 5.8</td>
<td>7.6</td>
</tr>
<tr>
<td>Enhydra fluctuans</td>
<td>&gt; 1000</td>
<td>525 ± 5.32</td>
<td>&gt;1.90</td>
</tr>
<tr>
<td>Moringa oleifera</td>
<td>724.5 ± 4.12</td>
<td>74.8 ± 4.7</td>
<td>9.67</td>
</tr>
<tr>
<td>Morus alba</td>
<td>604.2 ± 3.7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Odina wodier</td>
<td>336.5 ± 4.84</td>
<td>22.4 ± 3.6</td>
<td>15.02</td>
</tr>
<tr>
<td>Piper beetle</td>
<td>792.8 ± 5.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Stereospermum suaveolens</td>
<td>664.6 ± 3.29</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>130 ± 3.8</td>
<td>2.1 ± 0.5</td>
<td>61.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> 50% cytotoxic concentration for Vero cells in μg/ml.

<sup>b</sup> Concentration of test agent (μg/ml) producing 50% reduction of virus induced plaques.

<sup>c</sup> Selectivity index (SI)=CC<sub>50</sub>/EC<sub>50</sub>.

ND, Not detectable.

Table 3—Bioactivity of selected plants with the major compounds isolated so far (contd.)

<table>
<thead>
<tr>
<th>Plant name (Family)</th>
<th>Bioactivity of extract(s)</th>
<th>Compounds isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegle marmelos (Rutaceae)</td>
<td>Crude extracts showed antimicrobial, antiinflammatory, antipyretic, radio-protective, anti-spermatogenic, anti-hyperlipidemic, antiulcer, analgesic, anti-diabetic, antioxidant, anticancer activity&lt;sup&gt;20&lt;/sup&gt;.</td>
<td>Aegelin, Skinminiane, Lupeol, Cineole, Citral, Citronellal, Cuminaldehyde, Eugenol, Fagarine, Marmelide, Marmesinin, Marmelosin, Marmin, luvangetin, Aurapten, Psoralen and tannin.</td>
</tr>
<tr>
<td>Butea monosperma (Fabaceae)</td>
<td>Used as tonic, astringent, aphrodisiac and diuretics. Roots are used in filariasis, helminthiasis, piles, ulcer, night blindness; have anti-tumour, antifertility, aphrodisiac, analgesic activities. Flowers help in diarrhoea, and used as astringent, diuretic, and tonic.</td>
<td>2′-hydroxy genistein, Biochanin A, Butein, Butrin, Cajunin, Calycosin, Coreispin, Daídzein, Docosanoic acid, Flemmichapparin C, Formonentin, Hypogallic acid, Lupeol, Lupeonone, Medicarpin, Medicarpin, Monospermoside, Plastron, Prunetin, Sulphuresin, and Trihydroxy-flavone.</td>
</tr>
<tr>
<td>Dillenia indica (Dilleniaceae)</td>
<td>Fruit extract have anticancer activity against human leukemic cell lines (U937, HL60, K562); anti-diabetic ability in alloxan-induced diabetic Wistar rats; and cholesterol lowering or anti-hyperlipidemic activity in STZ-induced diabetic rats&lt;sup&gt;18&lt;/sup&gt;.</td>
<td>3b-hydroxylupane-13b-28-lactone, Betulin aldehyde, Betulin, Betulinic acid; dihydrokaempherol, Dillenitin, Gallic acid, Isohamnetin, Kaempferol, Lupeol, Myricetin, Naringenin, Quercetin, and Sitosterol.</td>
</tr>
<tr>
<td>Enhydra fluctuans (Asteraceae)</td>
<td>Have anti-oxidant, anti-cancer, anti-diarrhoeal, analgesic, phagocytic, hepatoprotective, neuroprotective and cytotoxic activity&lt;sup&gt;21&lt;/sup&gt;.</td>
<td>Enhydrin, Fluctuandin, Fluctuanin, Germanacanolide, Gibberellin A13, Gibberellin A9 and β-carotene, Aurantiamide acetate, Benzyl isothiocyanate, Chlorogenic acid, 4-(α-L rhamnopyranosyloxy) benzyl glucosinolate, 4-(α-L rhamnopyrano-syloxy) benzyl isothiocyanate, Moringinine, Niazimicin, Niaziminin, Pterygospermin, 4-(4′-O-acetyl-α-L-rhamno-pyranosyloxy) benzyl isothio-cyanate, and Quercetin.</td>
</tr>
<tr>
<td>Moringa oleifera (Moringaceae)</td>
<td>Various parts are reported to have antimicrobial, anti-cancer, anti-hyperglycaemic, dyslipidemia, anti-oxidant and anti-inflammatory activities&lt;sup&gt;23&lt;/sup&gt;.</td>
<td></td>
</tr>
</tbody>
</table>

(contd.)
detectable anti-viral activity in all the three widely used assay system. Based on the selectivity index (SI), the preferential antiviral activity (EC\textsubscript{50}) of the test extracts in relation to its cytotoxicity (CC\textsubscript{50}), the extracts of \textit{D. indica}, \textit{M. oleifera} and \textit{O. wodier} were found to be more effective (Table 3) and thus, need to be tested on other herpes viruses along with their chemical profiling and possible mode of action for the management of infections caused by HSV-1.

**Discussion**

The development of new antiviral agents from traditional medicines, capable of inhibiting herpes virus infection, represents an attractive strategy, particularly in immune-compromised individuals and neonates, who often generate ACV-resistant HSV strains. In our continued quest for identifying new leads from ethnomedicinal plants of traditional use we have evaluated nine plant extracts, selected on the basis of their use in skin, intestinal and sexual ailments. The cytotoxicity study revealed that the crude methanol extracts of the test plants had different CC\textsubscript{50} due to the variable concentration of bioactive compound(s) present within those plant extracts, and the antiviral dose was far below their cytotoxic concentrations. The antiviral activity study, using three different test systems like CPE reduction, MTT and PRA, showed that five extracts, namely \textit{Butea monosperma}, \textit{Dillenia indica}, \textit{Enhydra fluctuans}, \textit{Moringa oleifera} and \textit{Odina wodier} had detectable antiviral activity compared to the drug of choice acyclovir, as they effectively inhibit HSV-1 infection in Vero cells without reducing cell viability. Interestingly all these five extracts also inhibited the growth of the human isolate VU-09, isolated from a patient infected with HSV-1, indicating that these plant extracts need to be studied further with other viruses of herpes virus family.

The results of this study provide evidence that the Ethnopharmacology can also be a guide for the screening of biologically active plant materials against viral infections. Here, we have used 100 % inactivation of the viral strains to define the antiviral activity of an extract, and found that some extracts had partial antiviral activity, probably due to their varied chemical nature. Out of nine plant extracts tested, \textit{D. indica}, \textit{M. oleifera} and \textit{O. wodier} exhibited potent anti-HSV activity; of which \textit{D. indica} and \textit{O. wodier} are traditionally used in skin infections. One of the reasons for search of new antivirals from ethnomedicinal plants is the wide acceptability of traditional medicines and their relatively low toxicity profile. Secondly, the widely used antiviral drug acyclovir neither able to eliminate the virus from the host body, nor to prevent the recurrent infections\textsuperscript{1,3}. Moreover, as ACV and related analogues

### Table 3: Bioactivity of selected plants with the major compounds isolated so far

<table>
<thead>
<tr>
<th>Plant name (Family)</th>
<th>Bioactivity of extract(s)</th>
<th>Compounds isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Morus alba} (Moraceae)</td>
<td>Root extract possesses antidiabetic; while leaf extract have antimicrobial, antioxidant, anticancer anti- atherosclerosis, immuno-modulating, anti- inflammatory, anti-depressant, neuro- and hepato-protective activities. Bark (root and shoot) extracts have skin toning, uricosuric and nephroprotective effect\textsuperscript{24}.</td>
<td>Acetyl-amyrin, Albano B, 7, 2', 4', 6-tetrahydroyox-6-geranylflavanone, Cyclomorusin, Eudraflavone B hydroperoxide, Kuwanon S, Leachianone G, Moralbanone, 1-deoxyyirimycin, Mulberrofuran G, Mulberrosid C, and Oxydihydromorusin.</td>
</tr>
<tr>
<td>\textit{Odina wodier} (Anacardiaceae)</td>
<td>Leaves and stem bark possesses anti-inflammatory, antiallergic, anti-oxidant, and anti-diarrhoeal activity\textsuperscript{25,28}.</td>
<td>Anthranol B, Chlorogenic acid, Ellagic acid, Leucocyanadin, Leucodelphinadin, Physcion, Quercetin, Quercetin-3-arabinoside, Rutin, and β-sitosterol.</td>
</tr>
<tr>
<td>\textit{Piper betle} (Piperaceae)</td>
<td>Leaf extract showed antimicrobial, antioxidant and anti-inflammatory activities in high concentration. Used in type 2 diabetes, infertility, skin infections (dermatophytosis), and to potentiate estrogen. Leaf extract protect liver from CCl\textsubscript{4} induced damage with antidepressant activity\textsuperscript{29}.</td>
<td>Allypyrocatechol diacetate, Campene, Caryophyllene, Chavibetol, Eugenol, Flavonoids, Hydroxynovacil, Polyphenol, Saprobe, Ursolic acid, α-Pinene, α-tocoerpoterol, and β-Carotene.</td>
</tr>
<tr>
<td>\textit{Stereospermum suaveolens} (Bignoniaceae)</td>
<td>Root and leaf decoction have antipyretic activity; while the bark extract exhibited antibacterial, anti-tubercular, neuroprotective, antiulcer, gastro and hepatoprotective potential; but Lapachol of bark showed hypoglycaemic and anticancer activity\textsuperscript{27,28}.</td>
<td>Beta-sitosterol, Ceryl alcohol, Cyclooolivil, Dehydrocetol, Dihydro-alpha-lapachone, Lapachol, Oleic acids, Palmitic acid, Stearic acid, Stereolsin, Triacontanol and Triacontan.</td>
</tr>
</tbody>
</table>
targets the thymidine kinase enzyme and or DNA polymerase of herpes viruses, its extensive and long-term use yielded frequent drug-resistant strains due to mutations in viral thymidine kinase and/or DNA polymerase by altering the substrate sensitivity. Additionally, the efficacy of therapeutic vaccines against primary and recurrent HSV infection has failed and thus, search for natural alternative is the top priority to control and prevent HSV infections and its transmission. Our results indicated that the elucidation of active constituents and the possible mode of action of those plant extracts showing promising anti-HSV activity may provide useful lead for the development of effective antiviral agents.

Acknowledgement
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References


