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ABSTRACT

PHARMACOLOGICAL SCREENING OF *MEMECYLON SISPARENSE* GAMBLE FOR ITS ANTI-MICROBIAL, ANTI-OXIDANT AND ANTI-CANCER ACTIVITIES

Memecylon sisparense Gamble (MSG) belongs to melastomataceae family, having wide range of pharmacological activities like antioxidant, hepatoprotective, anti-inflammatory, anti-diabetic etc. For the first time, this study is aimed towards identification of biologically active compounds in MSG leaf ethyl acetate extract (MSGLEAE) by GC-MS analysis along with molecular docking studies for confirming the biological activity of identified compounds. The antioxidant, anticancer potential of the MSGLEAE was studied by *in vitro* and *in vivo* models.

Out of 41 compounds identified, 20 were found having biological activities like nephroprotective, anti-cancer, antioxidant, antibiotic, hepatoprotective, inhibition of uric acid production etc. The identified compounds were docked against the respective protein structures for cardioprotective, nephroprotective, anticancer activity.

MSGLEAE has shown good antioxidant activity along with anti-bacterial activity having highest zone of inhibition on *Staphylococcus aureus* followed by *S. epidermidis* then *Pseudomonas aeruginosa*, *Eschericia coli* followed by *Bacillus subtilis*, *B. cereus* with lowest zone of inhibition respectively.

Swiss albino male mice were treated with MSGLEAE (250, 500 mg/kg, *p.o*) for nine consecutive days against doxorubicin (DOX) (15 mg/kg, *i.p*) and cisplatin (12 mg/kg, *i.p*) on seventh day and evaluated for cardioprotective, nephroprotective activity.

In Doxorubicin induced cardiotoxicity, the changes in heart tissue were assessed from ECG recording in which MSGLEAE decreased the ST segment which was elevated in DOX treated animals. In biochemical estimation of CK-MB, LDH levels found to be increased significantly in DOX group by comparing to control group. Cisplatin induced nephrotoxicity is identified by an increase in serum blood urea nitrogen and creatinine which was decreased in the extract pre-treated groups. In cardiac and renal tissue, MSGLEAE @ 500 mg/kg decreased MDA, NO markers with an increase in SOD, CAT and GSH levels thereby showing protective mechanism against doxorubicin and cisplatin induced oxidative stress, evident by histopathological studies.

Out of the 6 cell lines screened for cell viability assay, MSGLEAE has shown IC₅₀ of 48.40 ± 1.68 µg/ml in MDA-MB-231 cells. The combination of MSGLEAE along with DOX also

had shown synergistic effect. MDA-MB-231 cells treated with MSGLEAE had shown cell cycle arrest in SubG1 phase along with an increase in late apoptotic stage in Annexin V apoptotic assay with an increase in concentration and supported by western blot analysis.

Nude mice were implanted with MDA-MB-231 cells, after tumor volumes reached palpable size, randomly grouped and treated with 500 mg/kg MSGLEAE where as combination group was treated with DOX 0.5 mg/kg, *i.p* weekly twice along with MSGLEAE daily 250 mg/kg, DOX group treated with 1 mg/kg, *i.p* weekly twice for 28 days. The tumor volumes was decreased significantly in MSGLEAE, DOX, combination treated groups by comparing to tumor control group. The bodyweights in MSGLEAE, combination group were retained where as in DOX treated group, there was a decrease in bodyweight was observed. In combination group, tumor volumes also decreased when compared to DOX group by retaining the body weights of xenografts mice, supported by Immunohistochemistry.

This is the first ever report in terms of the antioxidant potential of MSGLEAE. Pre-treatment with MSGLEAE has a significant therapeutic benefit during DOX and CP therapy by inhibiting oxidative stress through inhibiting lipid peroxidation, enhancing the antioxidant activity. Therefore *Memecylon sisparens* Gamble leaf may find a role in organoprotective activity by preventing the oxidative stress caused by DOX, CP along with its anticancer activity.

KEYWORDS: *Memecylon*, Phytochemicals, antioxidant, breast cancer

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LIST OF SYMBOLS AND ABBREVIATIONS	
%	Percentage
° C	Degree centigrade
µg	Microgram
µl	Microliter
µm	Micrometer
•O ²⁻	Superoxide radical
•OH	Hydroxyl radical
ADP	Adenosine di phosphate
ATP	Adenosine triphosphate
ATCC	American type culture collection
Cm	Centimeter
Conc	Concentration
Cu	Copper
Dl	Deciliter
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EAE	Ethyl acetate extract
Fe	Iron
FRSA	Free radical scavenging activity
G	Gravity
GAE	Gallic acid equivalent
GC-MS	Gas chromatography- Mass spectroscopy
Gm	Gram
H	Hour
H ₂ O ₂	Hydrogen peroxide
Hg	Mercury
HPTLC	High performance thin layer chromatography
M	Meter
M	Molar
MBC	Minimal bactericidal concentration
MIC	Minimal inhibitory concentration
MAPKs	Mitogen-activated protein kinases
MSGLEAE	<i>Memecylon sisparens</i> Gamble leaves ethylacetate extract
MTCC	Microbial type culture collection
Mg	milli gram
Min	Minutes

MI	milli litre
Mm	milli meter
Mn	Manganese
NF-κB	nuclear factor κ light-chain-enhancer
Nm	Nanometer
NOS-2	Nitric oxide synthase 2
O ₂	Oxygen
OH ⁻	Hydroxyl anion
OECD	Organisation for Economic cooperation and Development
Pb	Lead
PDB	Protein Data Bank
PUFA	Polyunsaturated fatty acids
RE	Rutin Equivalent
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SOD	Super oxide dismutase
UV	Ultra Violet
V	Volts
w/v	Weight/ volume