SYNERGISTIC EFFECT OF BIOACTIVE COMPOUNDS OF POLYHERBAL FRACTIONS OF *CLINACANTHUS NUTANS* AND *ELEPHANTOPUS SCABER* IN THE TREATMENT OF WOUND

by

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LIST OF ABBREVIATIONS

ATR	Attenuated Total Reflection Method
CID	Collision-induced Dissociation
CN	*Clinacanthus nutans*
DB	Database
EGF	Epidermal Growth Factor
ES	*Elephantopus scaber*
ECC	Extracted Compound Chromatogram
ESI-MS	Electrospray ionization-tandem mass spectrometry
ECM	Extracellular Matrix
FGF	Fibroblast Growth Factor
FTIR	Fourier Transform Infrared
HPLC	High-Performance Liquid Chromatography
IR	Infrared
IG	Indukantha Ghritha
IL	Interleukins
ICH	International Council for Harmonisation
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
MFE	Molecular Feature Extraction
IC50	Median Effective Concentration

NMR	Nuclear Magnetic Resonance Spectroscopy
PDGF	Platelet-derived growth factor
PHF	Polyherbal Formulation
tR	Retention Time
RA	Rheumatoid Arthritis
Rf	Retention Factor
RDA	Retro-Diels-Alder
ROS	Reactive Oxygen Species
SEM	Standard Error Measurement
TGF-β	Transforming Growth Factor-beta
TNF-α	Tumor Necrosis Factor Alpha
TIC	Tentative Identification of Compound
TLC	Thin-layer Chromatography
UV	Ultraviolet
DPPH	2,2-diphenyl-1-picrylhydrazyl

Kesan Sinergistik Sebatian Bioaktif dari Campuran Herba *Clinacanthus nutans* dan *Elephantopus scaber* Dalam Merawat Luka

ABSTRAK

Perubatan moden telah mengenal pasti bahawa perubatan herba sebagai suatu bentuk perubatan alternatif. Setiap orang dalam kehidupan sehariannya akan mengalami pelbagai jenis luka. *Clinacanthus nutans* dan *Elephantopus scaber* terkenal sebagai penyembuh luka dalam rawatan herba tradisional. Penyelidikan ini dijalankan untuk formulasi polyherba daripada daun *Clinacanthus nutans* dan *Elephantopus scaber* dalam rawatan penyembuhan luka. Seterusnya, bahan bioaktif dikenal pasti daripada berlainan bahagian menggunakan kromatografi dan kaedah spektroskopi. Bahagian yang larut air telah dipisahkan lagi menggunakan kromatografi untuk mengasingkan bahan bioaktif. Campuran herba *Elephantopus scaber* dan *Clinacanthus nutans* telah dinilai sebagai antioksida secara in-vitro dengan aktiviti memerangkap radikal oleh DPPH diikuti dengan pemisahan formulasi herba bagi aktiviti penyembuhan luka secara in-vivo menggunakan model yang berbeza. Hasilnya menunjukkan bahawa ekstrak mentah *Elephantopus scaber* berdasarkan EC_{50} menunjukkan tindakbalas yang lebih cepat tetapi kurang peratusan perencatan berbanding formulasi poliherba baru daripada ekstrak mentah. Formulasi poliherba mempunyai peratusan perencatan tertinggi (89.49%) pada dos yang sama berbanding *Elephantopus scaber* secara individu (87.66%). Perbandingan antara semua ekstrak mentah dan formulasi poliherba baru mendapati bahawa pecahan etil asetat dalam formulasi poliherba mempunyai aktiviti tertinggi (EC_{50} 14.83µg/ml) dengan % perencatan (89.28%). Seterusnya semasa penilaian penguncupan luka pada model luka untuk pemotongan dan pembedahan, pecahan etil asetat mempunyai aktiviti tertinggi dengan ($P<0.001$) dan ($P<0.0001$) masing-masing. Semasa model luka terbakar pecahan akuas ($P<0.001$) mempunyai aktiviti tertinggi diikuti dengan pecahan etil asetat ($P<0.0001$). Analisis cecair kromatografi spektrometri jisim (LCMS) telah mengenal pasti kewujudan beberapa sebatian berasakan flavonoid yang bertindak secara sinergistik dengan sebatian bioaktif. Seterusnya kewujudan sebatian bioaktif disahkan oleh cecair kromatografi pengionan elektrospray- seiring dengan spektroskopi jisim (LC-ESI-MS/MS). Sesetengah dari sebatian bioaktif dapat dikenalpasti sebagai apigenin, luteolin, b-sitoterol, 3'-hydroxy-5,6,7,4'-tetramethoxyflavone, quercetin, vanillin, asid gallik, asid benzoik dan rutin dan disahkan menggunnakan cecair kromatografi berprestasi tinggi. Daun-daun *Clinacanthus nutans* dan *Elephantopus scaber* diekstrak menggunakan 50% etanol (EtOH) dan dipisahkan menggunakan gel silika, Sephadex LH-20 untuk mengasingkan sebatian asli. 2-(aminomethylsulfinyl)-N-hydroxyacetamide adalah sebatian yang di cadanghan dapat diasingkan dari pecahan akuas formulasi poliherba. Pencirian sebatian tercapai dengan menggunakan IR dan LCMS pada pecahan akuas. Kesimpulannya, flavonoid meningkatkan aktiviti anti-oksidan yang mempercepatkan kadar penguncupan luka dan bertindak balas secara sinergistik dengan sebatian bioaktif lain.

Synergistic Effect of Bioactive Compounds of Polyherbal Fractions of *Clinacanthus nutans* and *Elephantopus scaber* in the Treatment of Wound

ABSTRACT

Modern healthcare system recognizes herbal medicine as a form of alternative medicine. Everyone in their life span experiences a different kind of wound. *Clinacanthus nutans* and *Elephantopus scaber* are well known traditional wound healing herbs. The present research work develops a new polyherbal formulation from the leaves of *Clinacanthus nutans* and *Elephantopus scaber* in the treatment of wound. Furthermore, bioactive compounds from different fractions were identified by using chromatography and spectroscopic method. The water soluble was further fractioned to isolate bioactive compounds. The herbal combination of *Elephantopus scaber* and *Clinacanthus nutans* were evaluated as an in-vitro antioxidant activity containing equal amount of both medicinal herb with their individual herbal activity followed by fractionation of polyherbal formulation for in-vivo wound healing activities. Antioxidant activity was performed in vitro by DPPH radical scavenging antioxidant activity followed by in-vivo wound healing activities using different wound model. The leaves of *Clinacanthus nutans* and *Elephantopus scaber* were extracted with 50% ethanol (EtOH) and separated using silica gel, Sephadex LH-20 to isolate the pure compounds. 2-(aminomethylsulfinyl)-N-hydroxyacetamide was the proposed compound isolated from aqueous fraction of the polyherbal formulation. The characteristics of the isolated compounds were achieved using Infrared spectroscopy (IR) and LCMS scan on aqueous fraction. The result showed that *Elephantopus scaber* crude extract on the basis of EC_{50} performs a much faster action but with less % inhibition as compared to the combination of the new polyherbal formulation of crude extract. The polyherbal formulation has the highest % inhibition (89.49%) at the same dose as compared to individual *Elephantopus scaber* (87.66%). Comparison among all crude and fractions of new polyherbal formulation, it was found that the ethyl acetate fraction of polyherbal formulation has the fastest activity, EC_{50} 14.83 µg/ml, with inhibition of 89.28%. Furthermore, during evaluation of wound contraction on excision and incision wound model, ethyl acetate fraction possess highest activity with ($P < 0.001$) and ($P < 0.0001$) respectively. During burn wound model, aqueous fraction ($P < 0.001$) possesses highest activity followed by an ethyl acetate fraction ($P < 0.0001$). Liquid chromatography-Mass spectrometry (LCMS) analysis identifies the presence of several flavonoid-based compounds that work synergistically with other bioactive compounds. These bioactive compounds were further confirmed by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS). The prescence of bioactive compounds such as apigenin, luteolin, b-sitosterol, 3'-hydroxy-5,6,7,4'-tetra methoxy flavone, quercetin, vanillin, gallic acid, benzoic acid and rutin were confirmed by using High performance liquid chromatography. In conclusion, flavonoid increases the antioxidant activity that surge the rate of wound contraction and work synergistically with other bioactive compounds.