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DETERMINATION OF CHEMICAL COMPOSITION, TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND *IN-VITRO* ANTIOXIDANT CAPACITY OF VARIOUS ORGANIC EXTRACTS OF *GYNURA CREPIOIDES* LEAVES CULTIVATED IN MALAYSIA

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Keywords:

Chemical composition, Antioxidant, *Gynura crepioides*, Phenolic, and flavonoid content, Crude extracts

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ABSTRACT: *Gynura crepioides*, also known as Okinawa Spinach, belongs to the family Asteraceae, which is known to possess various medicinal properties. The present study was thus aimed at determining the GC-MS based chemical profiling, total phenolic content (TPC), total flavonoid content (TFC) and anti-oxidant (AO) capacity of the organic crude extracts such as hexane, chloroform-ethyl acetate, obtained from the extraction of the leaves of *G. crepioides* by application of Folin-Ciocalteu method, aluminium chloride calorimetric method and DPPH radical scavenging assay, respectively. The results obtained from UV-VIS microplate spectrophotometer indicated a high amount of phenolic and flavonoid content in the leaves. Ethyl acetate extract was found to display highest TPC (10.09 ± 0.20 mg GAE/g) and TFC (9.56 ± 0.22 mg QE/g). Interestingly, ethyl acetate extract also displayed highest AO capacity with IC_{50} value of 0.74 mg/mL as compared to chloroform and hexane extracts with IC_{50} values of 0.90 mg/mL and 1.50 mg/mL, respectively with respect to the standard BHT (IC_{50}=0.04 mg/mL). GC-MS revealed the presence of sulfurous acid, 2-ethylhexylisohexyl ester (11.53%) as a major compound in hexane extract, stigmasterol (13.80%) in chloroform extract and squalene (21.32%) in ethyl acetate extract, respectively. The results suggested that *G. crepioides* has the potential to be a source of alternative medicine due to its reportedly good anti-oxidant properties.

INTRODUCTION: There has been of serious concern about the usage of plant-based drugs. A big ratio of the population still believes and depends on traditional and herbal medicines for their primary healthcare needs. Hence, the need to explore the use of some medicinal plants as alternatives for similar pharmacological effect is of the essence.

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Gynura crepioides, also known as hung-tsoi or red vegetable is an important member of the Asteraceae family which is indigenous to Indonesia but is also cultivated in Japan, Malaysia, and India. It is a perennial plant that prefers a warm and wet climate.

The 2 feet tall plant thrives in subtropical and tropical areas. It has a soft branch, and the leaves are edged lance-like, which are green on both sides or can be purple underneath **Fig. 1**. In the markets of Taiwan, the shoots of Okinawan spinach are sold as a vegetable, and it is also used to give a red coloring to foods, such as rice. Malaysia has about 7411 plant species with pharmaceutical and

medicinal properties1and approximately 1200 plant species in Malaysia are reported to possess therapeutic characteristic and have been used in traditional treatment [2]. Plant extracts that are either pure compounds or standardized extracts are natural products that have limitless potential for new drug discoveries because of their unmatched availability of chemical diversity [3]. Traditional medicines that come from plants consist of a wide range of bioactive substances that could be used in the treatment of chronic as well as infectious diseases [4] for example alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more [5].

FIG. 1: PLANT OF *GYNURA CREPIOIDES*

Phenolic compounds are secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenylpropanoid pathways in plants [6]. Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds 7 **Fig. 2**. Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, anti-atherogenic, anti-inflammatory, antimicrobial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [8, 9, 10, 11, 12].

The beneficial effects derived from phenolic compounds have been attributed to their antioxidant activity. Flavonoids, on the other hand, constitute the largest group of plant phenolics, accounting for over half of the eight thousand naturally occurring phenolic compounds [13]. Essentially their structure consists of two aromatic rings, which are joined together by an intermediate 3-carbon bridge, usually in the form of a heterocyclic ring bearing an oxygen atom **Fig. 3**.

Substitutions to rings these two aromatic side rings give rise to different compounds within each class of flavonoids [14]. These substitutions may include oxygenation, alkylation, glycosylation, acylation, and sulfation [15]. Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate [16]. Antioxidants can be synthesized *in-vivo* (*e.g.*, reduced glutathione (GSH), superoxide dismutase (SOD), *etc*.) or taken as dietary antioxidants [16, 17].

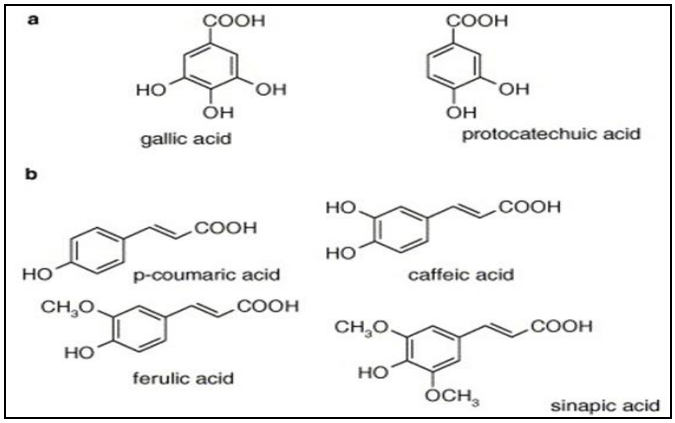

FIG. 2: EXAMPLE OF SOME PHENOLIC COMPOUNDS

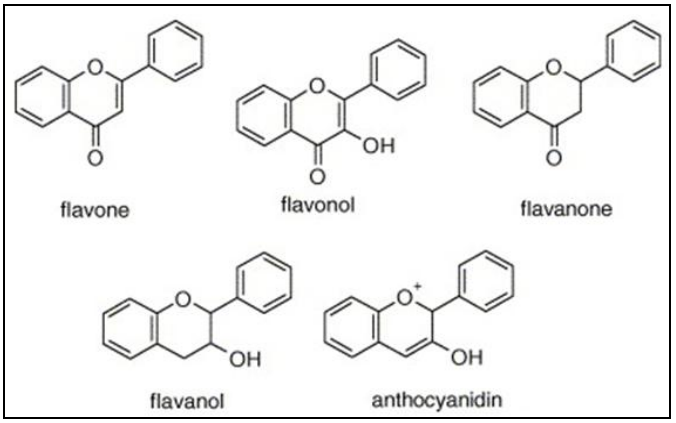

FIG. 3: GENERIC STRUCTURE OF MAJOR CLASSES OF FLAVONOIDS

Plants have long been a source of exogenous (*i.e.*, dietary) antioxidants. It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential [18]. The interest in the exogenous plant antioxidants was first evoked by the discovery and subsequent isolation of ascorbic acid from plants [19]. Since, then, the antioxidant potential of plants has received a great deal of attention, because increasing oxidative stress has

been identified as a major causative factor in the development and progression of several life-threatening diseases, including neurodegenerative and cardiovascular disease. It is undeniable that many studies regarding the TPC, TFC and AO activity of different parts of various medicinal plants have been carried out, but it is important to mention here that there is no specific study done on the leaves of *G. crepioides*. Plants with high phenolic and flavonoids are reported to be a source of natural antioxidants. Therefore, the aim of the present study was, to determine the chemical composition, TPC, TFC, and AO capacity of various organic extracts obtained from the leaves of *G. crepioides*.

MATERIALS AND METHODS:

Chemicals, Reagents, and Instruments: Follin-Ciocalteu phenol reagent, BHT, 2,2-diphenyl-1-1 picrylhydrazyl, aluminum chloride, quercetin, potassium acetate, ascorbic acid, gallic acid and sodium carbonate (Na_2CO_3) were obtained from Sigma-Aldrich. Hexane, chloroform, ethyl acetate, and methanol were purchased from Merck. All chemicals were of analytical grade and used without any further purification. The GC-MS analysis was conducted on a Perkin Elmer Clarus 500, GC-MS spectrometer equipped with VF-5 MS fused silica capillary column of 30 m × 0.25 i.d. and 0.25 µm film thickness. Perkin Elmer Lamda 20 UV-VIS microplate spectrophotometer was used to measure the absorbance for the determination of AA, TPC, and TFC of all the crude extracts.

Sample Collection: The fresh leaves of *G. crepioides* were collected at the local market at Tuaran district in Kota Kinabalu, Sabah at approximately 3 kg wet mass.

Sample Preparation: Fresh plant (3 kg) was washed with distilled water and shade dried at room temperature for 1 day to obtain 300 g of dry sample, which was powdered coarsely by the help of blender before solvent extraction.

Preparation of Organic Crude Extracts: Preparation of organic crude extracts was based on Khan *et al*., with some minor modification [20]. 300 g of the powdered sample was distributed equally among three beakers, having 400 ml methanol. The samples were left for 48 h in an orbital shaker, filtered and subjected to evaporation under reduced pressure to yield crude methanolic extract. The methanolic extract was then suspended with distilled water and transferred into a separating funnel. It was successively extracted by three different organic solvents with increasing polarity index, which was hexane, chloroform, and ethyl acetate, respectively. Finally, these filtrates were again evaporated under reduced pressure, and the organic crude extracts were collected.

Determination of Chemical Composition: The samples were prepared in ethyl acetate, chloroform, hexane and analyzed by injecting into GC-MS with a split injector at 300 °C. The VF-5 MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm) was employed. The temperature program was 50 °C, held for 10 min, increased at 3 °C/min to 250 °C, and finally hold for 10 min. Inert helium gas was employed as a carrier gas at a constant flow rate of 1.0 ml/min. The compounds were identified by comparison of their retention indices (RI) with those provided in the National Institute of Standards and Technology (NIST) library. Identification was assumed when a good match of RI was achieved.

Determination of Total Phenolic Content (TPC): Folin-Ciocalteu method was used to determine TPC in *G. crepioides*, taking gallic acid as the internal standard for the calibration curve [21]. 30 mg of gallic acid was dissolved in 30 ml methanol to obtain a concentration of 1 mg/mL. The resulting solution was further diluted by methanol to get the concentrations of 0, 20, 40, 60, 80, and 100 µg/mL. After 3 min, 200 µl of 10% Folin-Ciocalteu reagent and 800 µl of 700 mM of Na_2CO_3 were added in each concentration, and the solution was rested for 2 h in the dark at room temperature. Then after the absorbance was measured at a constant wavelength of 765 nm. 200 µl of methanol was used as blank control. 10% Folin-Ciocalteu, was prepared by dissolving 1.5 g of Na_2SO_3 in 20 ml of distilled water. Extracts samples were prepared by taking 30 mg of crude extract and diluting them with 30 ml of methanol. 200 µl of 10% Folin-Ciocalteu reagent and 800 µl of 700 mM of Na_2CO_3 were then added to it after 3 min, and the resulting solution was rested for 2 h in the dark at room temperature. 100 µl of these extracts and 1000 µl methanol was used as a control sample. The procedure was repeated in triplicate for all the three extracts, and the mean

value was calculated. The TPC was then calculated using the equation 1 and 2, respectively.

Phenolic Content: Gallic acid equivalent (GAE),

GAE = [Absorbance at 765 nm + C (from calibration curve)] / m.....1

Total Phenolic Content (mg GAE/g):

Total phenolic content (mg GAE/G) =mg GAE / Extract concentration (g).....2

Determination of Total Flavonoid Content (TFC): Aluminium chloride ($AlCl_3$) calorimetric method was used for the determination of TFC in *G. crepioides* with some minor modifications using quercetin as an internal standard [22]. Firstly, 30 mg of quercetin was dissolved in 30 ml methanol to obtain the concentration of 1 mg/mL which was further diluted by successive addition of methanol to get the series of 0, 20, 40, 60, 80 and 100 µg/mL of concentrations. Secondly, 360 µl of methanol and 24 µl of 10% $AlCl_3$ were added in each concentration followed by the addition of 24 µl of 1 M potassium acetate and 680 µl of distilled water.

The resulting solution was rested for 30 min in the dark at room temperature. The absorbance was then measured at a constant wavelength of 415 nm.

The 10% of $AlCl_3$, was prepared by dissolving 9.815 g of potassium acetate in 100 ml of distilled water. 120 µl of methanol and 182 µl of distilled water were used as blank control. Thirdly, for the samples, 30 mg of the crude extracts were dissolved in 30 ml of methanol and 360 µl of methanol and 24 µl of 10% $AlCl_3$ were added in 120 µl of these extracts. Then after, 24 µl of 1 M of potassium acetate and 680 µl of distilled water were added. The solution was left for 30 min in the dark at room temperature. 30 µl of plant extract and 272 µl of distilled water were used as control samples. Finally, the absorbance was measured at a constant wavelength of 415 nm using UV-VIS microplate spectrophotometer. The assay was repeated in triplicates, and the results were obtained by calculating the mean value. The TFC was calculated using equation 3 and 4.

Flavonoids Content: Quercetin equivalent (mg QE),

QE= [Absorbance at 415 nm + C (from calibration curve)] / m.....3

Total Flavonoid Content (mg GAE/g):

Total flavonoid content (mg GAE/g) = mg GAE / Extract concentration (g).....4

Determination of *in-vitro* Anti-oxidant Capacity by DPPH Radical Scavenging Assay: Antioxidant activity was tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [23]. A stock solution of DPPH was prepared by dissolving 2 mg of DPPH in 50 ml of methanol. The standard solution of BHT was prepared by dissolving 10 mg of BHT in 10 ml of methanol to obtain a concentration of 1 mg/mL. The standard BHT solution was further diluted by methanol to obtain a series of concentration of 0.01, 0.05, 0.10, 0.25, and 0.50 mg/mL. 585 µl DPPH was then added to each of these concentrations, and the mixture was shaken vigorously and left in the dark for 60 min at room temperature. Each of the test tube was covered with aluminum foil to prevent light degradation. The absorbance was measured at a constant wavelength of 517 nm by UV-VIS spectrometer.

A similar procedure was followed for the plant extracts just by replacing BHT with the extracts, and the concentrations were also changed to 0.125, 0.250, 500, 1000, 2000, and 4000 mg/mL. This assay is based on the ability of plant extracts to scavenge the stable DPPH radical, and it is observed by viewing the color change of DPPH from violet yellow. The assay was carried in triplicates, and the results were obtained as mean values. The interpretation of the results was based on the inhibition activity (I%). The percentage inhibition of free radical DPPH was determined using equation 5. IC_{50} value, which is defined as the concentration of substrate that causes 50% loss of the DPPH activity, was calculated from the regression equation for the concentration of extracts and the inhibition activity (I%) by equation 6.

Inhibition:

$I\% = (Absorbance_{control} - Absorbance_{sample} / Absorbance_{control} \times 100$.....5

IC_{50}: $y = mx + C$.....6

Statistical Analysis: Data were analyzed in three replicates for each treatment. With the help of Microsoft Excel, average values, means of variables, and the standard deviations (S.D.) were calculated to authenticate the significant differences.

RESULTS AND DISCUSSION: In this study, three crude extracts were obtained from the leaves of *Gynura crepioides*, which were hexane, chloroform, and ethyl acetate. For the extraction, the initial weight of the plant sample was 300 g. The percentage yield is calculated based on equation 7 **Table 1**. Selection of a suitable solvent for extraction is really important as it highly influences the quantity and quality of the final extract [24].

Percentage yield (%) = Weight of crude extract obtained / Weight of plant sample ×100.....7

TABLE 1: PERCENTAGE YIELD OF EXTRACTS

Sample	Weight of crude extract (g)	Percentage yield (%)
Hexane	7.89	2.63
Chloroform	2.43	0.81
Ethyl acetate	1.71	0.57

Chemical Components in the Hexane Leaf Extract of *Gynura crepioides*: GC-MS analysis of hexane-extracted to the identification of 9 clear peaks **Fig. 4**. The major chemicals constituents were sulfurous acid, 2-ethylhexylisohexyl ester (11.53%), decane, 2, 5, 9,-trimethyl- (8.53%), caryophyllene (7.90%), and tetradecanoic acid, 10, 13-dimethyl-, methyl ester (5.31%) **Table 2**. At the retention time (RT) of 11.55 min, GC-MS chromatogram showed the presence of tetradecane, 1-iodo which is an iodocompound [25]. Based on the research done by Thomas *et al*., and Shunmuga *et al*., tetradecane acid, 10,13-dimethyl-, methyl ester were reported, as a white crystalline fatty acid methyl ester, that possesses anti-insecticidal and anti-inflammatory properties in addition to hypo-cholesterolemic, antiandrogenic, antioxidant and nematicidehemolytic 5-alpha-reductase inhibition properties [26].

Chemical Components in the Chloroform Leaf Extract of *Gynura crepioides*: A total of 8 different compounds were obtained from the chloroform extract **Fig. 5**. The major compounds with highest peak area were reported to be stigmasterol (13.80%) followed by 5-Isopropyl-6-methyl-hepta-3, 5-dien, 2-ol (13.69%) **Table 3**. Stigmasterol is a steroid with antimicrobial, anti-cancerous, anti-inflammatory, anti-asthma, anti-arthritic and hepato-protective properties [27].

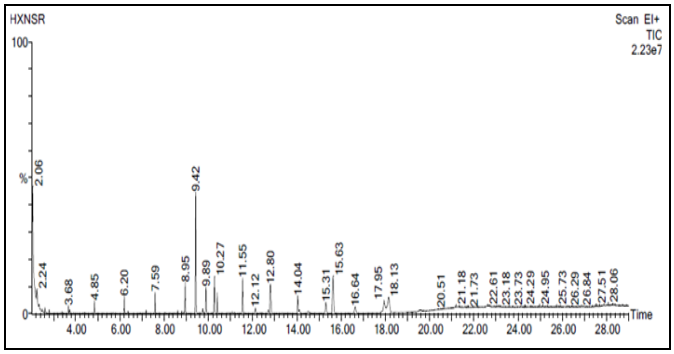

FIG. 4: CHROMATOGRAM OF HEXANE EXTRACT OF LEAVES OF *GYNURA CREPIOIDES*

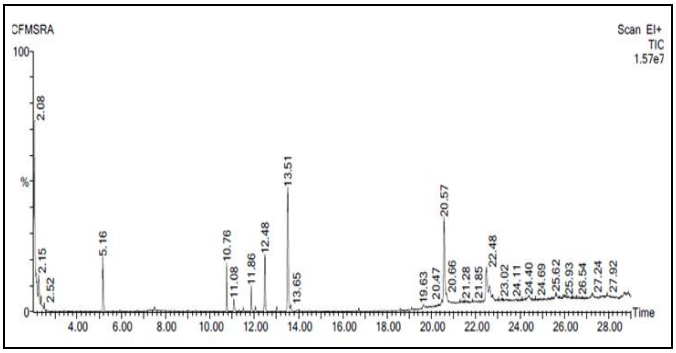

FIG. 5: CHROMATOGRAM OF CHLOROFORM EXTRACT OF LEAVES OF *GYNURA CREPIOIDES*

TABLE 2: CHEMICAL COMPOUNDS FROM HEXANE LEAF EXTRACT OF *GYNURA CREPIOIDES*

No.	Ret. Time	Name of compounds	Molecular formula	M.W.	Peak area (%)
1	6.20	Decane,6-ethyl-2-methyl	$C_{13}H_{28}$	184	6.61
2	7.59	Decane,2,5,9,-trimethyl-	$C_{13}H_{28}$	184	8.53
3	9.42	Caryophyllene	$C_{15}H_{24}$	204	7.90
4	10.28	Sulfurous acid, 2-ethylhexyl hexyl ester	$C_{14}H_{30}O_3S$	278	2.36
5	11.55	Tetradecane, 1-iodo	$C_{14}H_{29}I$	324	2.48
6	12.80	Sulfurous acid, 2-ethylhexyl isohexyl ester	$C_{14}H_{30}O_3S$	278	11.53
7	15.63	Tetradecanoic acid, 10,13-dimethyll-,methyl ester	$C_{17}H_{34}O_2$	270	5.31
8	17.95	18-Nonadecen-1-ol	$C_{19}H_{38}O$	282	6.51
9	18.15	Phytol	$C_{20}H_{40}O$	296	4.37

TABLE 3: CHEMICAL COMPOUNDS FROM CHLOROFORM LEAF EXTRACT OF *GYNURA CREPIOIDES*

No.	Ret. Time	Name of compounds	Molecular formula	M.W.	Peak area (%)
1	5.17	Phenylethyl alcohol	$C_8H_{10}O$	122	4.62
2	10.76	2(4*H*)-Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	$C_{11}H_{16}O_2$	180	3.20
3	11.08	Sulfur hexafluoride	F_6S	146	8.21
4	11.86	2,5-Dimethylhex-5-en-3-yn-2-ol	$C_8H_{12}O$	124	1.82
5	12.48	1-{2-[3-(2-Acetyloxiran-2-yl)-1,1-dimethylpropyl]cycloprop-2-enyl}ethanone	$C_{14}H_{20}O_3$	236	4.82
6	13.53	5-Isopropyl-6-methyl-hepta-3,5-dien,2-ol	$C_{11}H_{20}O$	168	13.69
7	20.57	Stigmasterol	$C_{29}H_{48}O$	412	13.80
8	22.48	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	$C_{15}H_{26}O$	222	6.47

Chemical Components in the Ethyl Acetate Leaf Extract of *Gynura crepioides*: GC-MS analysis of ethyl acetate extract led to the identification of 7 peaks under the same capillary conditions **Fig. 6**. The major components were 2, 6, 10, 14, 18, 22-tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl- which is commonly known as squalene (21.317%), followed by phytol (11.99%) **Table 4**. Squalene is reported to possess antibacterial, anti-sun tan, anti-tumor, anti-oxidant, perfumery, chemopreventive, immuno-stimulant, anti-pesticidal, lipoxygenase inhibitory properties [25].

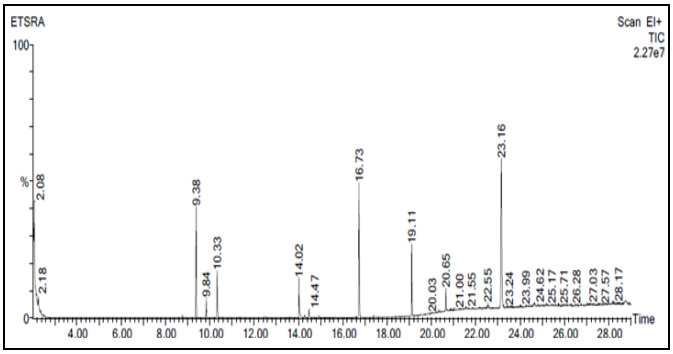

FIG. 6: CHROMATOGRAM OF ETHYL ACETATE EXTRACT OF LEAVES OF *GYNURA CREPIOIDES*

TABLE 4: CHEMICAL COMPOUNDS FROM ETHYLACETATE LEAF EXTRACT OF *GYNURA CREPIOIDES*

No.	Ret. Time	Name of compounds	Molecular formula	M.W.	Peak area (%)
1	9.39	Caryophyllene	$C_{15}H_{24}$	204	8.67
2	9.84	Bicyclo[3.1.1]hept-2-ene,3,6,6-trimethyl	$C_{10}H_{16}$	136	1.53
3	10.33	Phenol, 3,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206	4.46
4	14.02	Butanoic acid, 3,7-dimethyl-6-octenyl ester	$C_{14}H_{26}O_2$	226	3.84
5	16.73	Phytol	$C_{20}H_{40}O$	296	11.99
6	19.11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	5.64
7	23.16	Squalene	$C_{30}H_{50}$	410	21.32

Moreover, squalene, which is a triterpene, is also involved in the vitamin-D synthesis and helps reducing risk on cancer in humans [28]. On the other hand, phytol, which is a diterpene, possesses anti-microbial, anti-cancerous, anti-inflammatory, and diuretic properties [29, 30].

Estimation of Total Phenolic Content in the Leaves of *Gynura crepioides*: A phenolic compound is a class of antioxidant agent, which act as free radical terminators. Phenolic compounds exhibit considerable free-radical scavenging activity, which is determined by their reactivity as hydrogen- or electron- donating agents, their reactivity with other antioxidants and their chelating metal properties, as well as the stability of the resulting antioxidant-derived radicals [31]. As shown in **Fig. 7**, the TPC in ethyl acetate extract is highest (10.09 ± 0.20 mg GAE/g), followed by chloroform (9.22 ± 0.15 mg GAE/g) and hexane (4.96 ± 0.17 mg GAE/g). There is a difference of 50.83% of the TPC between ethyl acetate extract and hexane.

TABLE 5: TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS IN HEXANE, CHLOROFORM AND ETHYL ACETATE EXTRACTS OF *GYNURA CREPIOIDES* LEAVES

Plant extracts	Total phenolic contents (mg GAE/g)	Total flavonoid contents (mg QE/g)
Hexane	4.96 ± 0.17	4.76 ± 0.19
Chloroform	9.22 ± 0.15	8.47 ± 0.13
Ethyl acetate	10.09 ± 0.20	9.56 ± 0.22

Data are expressed as mean ± S.D. (n= 3).

Hence, it shows that there is a big gap in terms of TPC in these two extracts. The percentage difference of TPC ethyl acetate and chloroform

extract is 8.64%, which is relatively small as compared to ethyl acetate extract and hexane extract **Table 5**.

Estimation of Total Flavonoids Content in the leaves of *Gynura crepioides*: $AlCl_3$ was used for the determination of TFC. As shown in **Fig. 8**, the TFC is highest in ethyl acetate extract (9.56 ± 0.22 mg QE/g) followed by chloroform (8.47 ± 0.13 mg QE/g) and hexane (4.76 ± 0.19 mg QE/g).

The percentage difference of TFC between ethyl acetate and hexane is 50.23%, which is quite large whereas between ethyl acetate and chloroform is 11.38%, which is comparatively smaller **Table 5**.

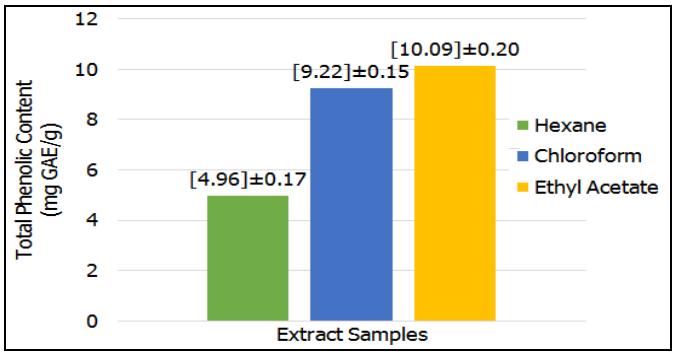

FIG. 7: TOTAL PHENOLIC CONTENT IN ORGANIC EXTRACTS. Data are expressed as mean ± S.D. (n= 3).

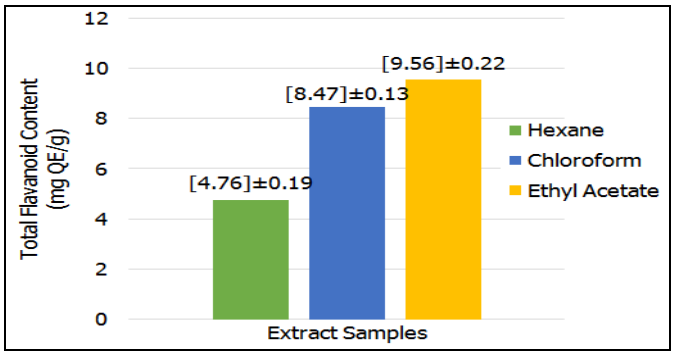

FIG. 8: TOTAL FLAVONOID CONTENT IN ORGANIC EXTRACTS. Data are expressed as mean ± S.D. (n= 3).

***In-vitro* Anti-oxidant Capacity of the Organic Extracts of *Gynura crepioides* Leaves:** DPPH assay is a robust method to determine the antioxidant potential of plant extracts [32].

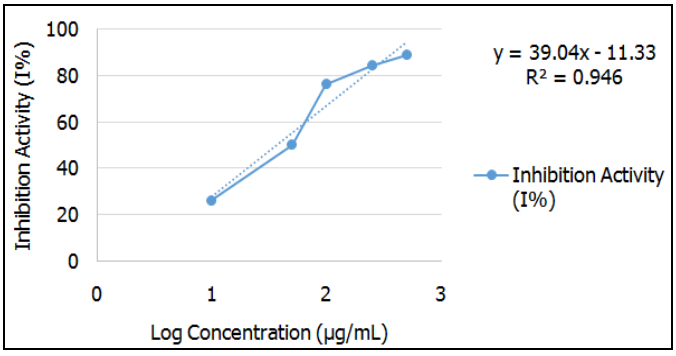

FIG. 9: INHIBITION ACTIVITY AGAINST LOG CONCENTRATION OF BHT

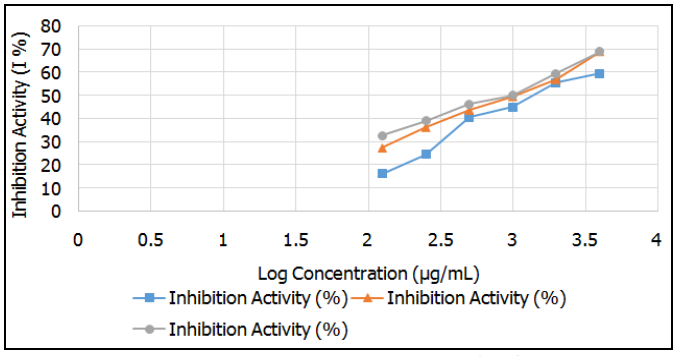

FIG. 10: PERCENTAGE INHIBITION (I%) AGAINST CONCENTRATION (µg/mL) OF SAMPLE EXTRACTS

This method is based on the reduction of methanolic DPPH solution in the presence of hydrogen donating compounds through the formation of non-radical compounds (DPPH-H). As the crude extract reduces the DPPH radical, the purple color of DPPH is bleached to yellow because of the increase of diphenyl-picrylhydrazine.

Fig. 9 shows the inhibition percentage of BHT while **Fig. 10** shows the inhibition percentage of the organic extracts. Based on the figures, there is not much percentage difference in the inhibition activities of each of the plant extract. For the ethyl acetate extract, the maximum value of the inhibition activity is 69.14% while in the chloroform extract the maximum value of inhibition activity is 69.05%, and for hexane extract, the inhibition activity is 59.60%.

As shown in **Table 6**, the plant extracts fared good to moderate as compared to the standard BHT. The lowest IC_{50} of 0.74 µg/mL was recorded for ethyl acetate. **Fig. 11** shows the graphical expression of IC_{50} values.

TABLE 6: IC_{50} VALUE OF BHT AND PLANT EXTRACTS

Sample	IC_{50} (mg/mL)
Hexane	1.50
Chloroform	0.90
Ethyl acetate	0.74
BHT	0.04

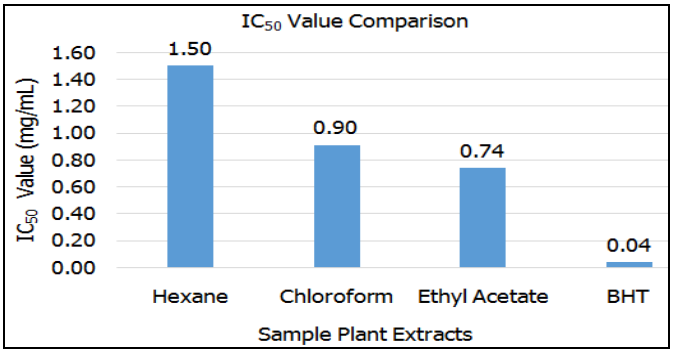

FIG. 11: IC_{50} VALUE OF BHT AND PLANT EXTRACTS

CONCLUSION: The leaves of *Gynura crepioides* were extracted by hexane, chloroform and ethyl acetate in the order of their increasing polarity and analyzed qualitatively *via* GC-MS to determine the chemical composition of each crude extract. In hexane extract, a total of 9 compounds, in chloroform extract a total of 8 compounds and in ethyl acetate extract a total of 7 compounds were identified. It was obvious from the results that the highest TPC, TFC and AO capacity was displayed by ethyl acetate crude extract, which is a strong polar solvent. It could also be suggested that phenolic and flavonoid content in the plants are a valuable contributor to the AO activity. Based on the antioxidant activity index (AAI), ethyl acetate extract (0.74 mg/mL) showed strongest antioxidant capacity followed by chloroform extract (0.90 mg/mL) and hexane extract (1.50 mg/mL). Based on these results of AAI, this plant could be considered as a good source of natural antioxidants.

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