Research Article

In vitro Evaluation of Purple Inflorescence of *Gomphrena globosa* (L.) Extracts for Anti-inflammatory Activity and its GC/MS Profile

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Submission Date: 13-02-2021; Revision Date: 15-04-2021; Accepted Date: 19-04-2021

ABSTRACT

The complex biological process that protects the body by triggering immune system for infection, injury or disease is called inflammation. When compared with acute inflammation, chronic inflammation lasts for weeks, months or years and cause tissue damage. Non-steroidal anti-inflammatory drugs play an important role in reducing pain and inflammation by blocking arachidonic acid metabolism through cyclooxygenase enzyme and thereby reducing the production of prostaglandins. Due to severe side effects associated with non-steroidal anti-inflammatory drug, many researchers are focusing on plant secondary metabolite with no or less side effects. In Trinidad, Globe Amaranth is used to treat several ailments for scientific validation of the folklore claim of this plant the present study was conducted. Decoction and hydroalcoholic flower extracts (pink varieties) of *G. globosa* was evaluated for its anti-inflammatory activity. Albumin denaturation method, anti-proteinase action, HRBC membrane stabilization and protein denaturation methods were carried out. Aspirin and diclofenac sodium were used as standard drug. The results showed that decoction extract inhibited heat induced protein denaturation ($p \leq 0.05$), inhibited proteinase action and heat induced hemolysis of erythrocyte at 500µg/mL. Hypo tonicity induced hemolysis was also found to be significant with 68.42% of inhibition ($p \leq 0.05$). The total phenol in decoction extract was 60mcg/mL and reducing power assay revealed significant antioxidant activity. The identification of bioactive compounds in hydroalcoholic extract was done using GC-MS. Earlier reports have showed that the compound 1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester possess antioxidant and anti-inflammatory activity. From the results, the decoction extract possess does dependent potent anti-inflammatory activity. This was plausibly due to phytoconstituents that acted individually or synergistically in exhibiting the potent activity.

Key words: Albumin, Cyclooxygenase, Anti-proteinase, Alkaloid, Prostaglandins, GC-MS, Hydroalcoholic, *Gomphrena globosa*, Phenol.

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INTRODUCTION

Prostaglandin is lipids synthesized from arachidonic acid by cyclooxygenase isoenzymes and plays an important role in inflammatory response.[1] Its mode of action is inhibited by nonsteroidal anti-inflammatory drugs. The pain and swelling of inflammation are mainly attributed by induction of COX-2 by cytokines that leads to prostaglandin production.[2] Acute inflammation is a complex internal beneficial process that occurs in response to pathogenesis, infection and injury.[1] Several anti-inflammatory drugs that inhibit COX-2 inhibitor is available in markets with known side effects for heart attack and stroke. Researchers are isolating bioactive compounds from plants to find potential COX-2 inhibitor as anti-inflammatory agent with no side effects.[3] COX-1 enzyme shows protectory role in gastrointestinal tract. COX-2 enzyme produces prostaglandins in higher rate and plays an important

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DOI: 10.5530/ajbls.2021.10.19

role in inflammation. One type of NSAID is COX-2 inhibitor that blocks COX-2 enzyme and their use is reported to side-effects such as stroke and heart attack.[3,4] The bioactive compounds in medicinal plants either individually or synergistically act on specific pathway, that can be used either as crude or pure compounds for treating several diseases including inflammations.[3] India is rich in diverse medicinal plants to treat various diseases. In recent years, especially during this COVID-19 people started using medicinal herbs in everyday life despite the progress in modern medicine. Plants such as *Achillea millefolium, Aconitum heterophyllum, Adhatoda vasica Nees, Aegle marmelos, Aloe vera, Azardirachta indica, Baccharis incarum, Bacopa Monnieri, Bonafousia sananho, Boswellia serrata, Bryophyllum pinnatum, Bursera simaruba, Caralluma thberculata, Cassia fistula, Cassia obtusifolia, Elephantophs scaber, Emblica officinalis, Erythrospermum monticoloum, Garcinia mangostana, Hammada elegans, Hedera rhombea, Iberis amara, Kirkia acuminata, Lantana camera, Lycopodium clavatum, Mikania cordata, Moringa olifera, Phyllanthus polyphyllus, Piper longum, Ricinus communis, Rheum australe, Saussurea costus, Sesbania sesban, S. chirata, T. Micrantha, T. diversifolia, T. lignosa, T. populnea, V. rosea, V. mocanera, X. spina, Z. africana* and *Z. officinalae*[5,6] are reported to have anti-inflammatory activity.

Genus *Gomphrena* is native to Central America and belongs to *Amaranthaceae* family that consists of 180 genera with 2500 species. This plant is cultivated as ornamental worldwide and has several folklore claims such as to treat cough, hemorrhage,[7] respiratory and reproductive problems,[8] cancer,[9] pain[10] and also known to possess cytotoxic, antimicrobial and cardiovascular activities, hypoglycemic activity.[10] The flowers of *G. globosa* were found to be richest source of betacyanins that can be used in food and cosmetic industries.[11] The phytoconstituents that are reported to possess anti-inflammatory activity are alkaloids, glycosides, terpenoids, flavonoids, polyphenols[12] resins, essential oils, polysaccharides, phenolic compounds, steroids, cannabinoids, fattyacids and plant glycoproteins.[13] As a scientific validation of the folklore claim of this plant the present study was conducted on *G. globosa* purple inflorescences to study the anti-inflammatory activity in decoction and hydroalcoholic flower extracts. This plant is being used as traditional folk medicine for treating acute and chronic inflammatory conditions. The bioactive compounds in *G. globosa* flower extracts were identified using Gas chromatography-mass spectrometry.

MATERIALS AND METHODS

Plant material and extraction

Gomphrena globosa flowers was collected from L.N. Puram region which is located in Pudukkottai District, Tamil Nadu, India, during the month of October to December, 2018. The collected plant was authenticated as *Gomphrena globosa* (L.) by Specimen No: CE 001 (Figure 1a and 1b).

Figure 1a: *Gomphrena globosa* (L.)

Figure 1b: Specimen No: CE 001.

For decoction preparation, 1g of fine powder was added to 200 mL of distilled water and boiled for 5 min. The mixture was allowed to stand at room temperature and then filtered. The filtered decoction was lyophilized and used for further assays.

The flower powder *G. globosa* was macerated with 70% ethanol (1:10) at 150 rpm for 1 hr. This hydroalcoholic extraction was filtered through Whatman No: 1 filter paper, evaporated and lyophilized. The lyophilized extracts were re dissolved in 70% ethanol (v/v) and stock solution of 20 mg/mL was prepared and used for further studies.

Qualitative analysis of Phytochemicals

Decoction and hydroalcoholic extracts of *G. globosa* were subjected to phytochemical analysis following standard method[14] for detection of alkaloid, flavanoid, terpenoid, sugar, proteins, saponins, gums and mucilages.

Assay of anti-inflammatory activity under *in vitro* condition

Inhibition of albumin denaturation assay

Different concentration (100 - 500µg) of flower extracts along with 1% of bovine serum albumin was incubated at 37°C for 20min and 37°C for 20min. After cooling, the turbidity was measured at 660 nm.[15] The percentage inhibition of albumin denaturation was calculated as follows:

$$\text{Inhibition (\%)} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \text{ X } 100$$

Anti-proteinase action assay

1ml of different concentrations of flower extract was mixed with 2ml of 0.06 mg of trypsin, 1 ml of 20 mM Tris-Hcl buffer and incubated at 37°C for 5 min. 1ml of 0.8% of casein was added and further incubated for 20 min. The reaction was stopped by adding 70% perchloric acid (2ml). The suspension was centrifuged and OD was measured in supernatant at 210nm.[16]

Preparation of blood sample and membrane stabilization

For human red blood cell membrane stabilization method, blood was collected from healthy individual and centrifuged at 3000 rpm for 10 min. The blood was washed with equal volume of normal saline till the supernatant was clear and colorless. The blood volume was measured and using normal saline 10% (v/v) suspension was reconstituted and used in following assays.

Hypotonicity induced haemolysis

Hyposaline (2ml), 0.5ml of human red blood cell suspension and 1ml of phosphate buffer was mixed with different concentration (100 - 500µg) of plant extracts. It was incubated at 37°C for 30min and centrifuged at 3000rpm. The hemoglobin content was estimated in the pellet at 560nm. Parvin *et al.* 2015[17] method was followed to measure hypotonicity induced hemolysis. Diclofenac sodium was used as a standard drug.[15] The membrane stabilization was calculated as follows:

Percentage protection = OD1−OD2/OD1×100

OD1 and OD2 – values (at 560nm) of hypotonic buffered saline solution and test sample respectively.

Inhibition of hemolysis by *G. globosa* extracts

In brief, 1 ml of test sample of different concentration (100 – 500µg/ml) was mixed with 1ml of 10% RBC suspension. In control tube saline alone was taken. The tubes were incubated at 56°C for 30min. Later, centrifuged and absorbance was read at 560nm.[18] The percentage inhibition of hemolysis was calculated as,

Inhibition (%) = Abs Control −Abs sample/Abs control × 100

Estimation of total phenol content

Folin-Ciocalteau method was used to measure the total phenolic content in the decoction and hydroalcoholic extracts of *G. globosa* using Gallic acid as a reference drug.[15,19] 2.25ml of Folin-Ciocalteau reagent was mixed with different concentrations of flower extracts, after 5 min 2.25 ml of sodium carbonate at a concentration of 6% was added and incubated at room temperature for 90 min. The OD values were obtained at 725nm. A calibration curve was drawn using gallic acid as standard.

DPPH-free radical scavenging assay

1ml of each flower extract, methanol and 0.3mM DPPH in methanol was taken, mixed and incubated in dark for 10 min in room temperature. OD was read at 517nm.[19,20] Scavenging activity was measured as,

Scavenging effect% = [(control absorbance–sample absorbance) / (control absorbance)] × 100

GC-MS analysis

The GC-MS analysis of hydroalcoholic extract of *G. globosa* was performed using GC-MS (Model - SHIMADZU QP2020). Helium (99.999%) was used as a carrier gas at a constant flow rate of 1ml/min and injection volume of 2µl (split ratio of 10:1) was employed. The GC-MS analysis time was 36 min. The unknown phytoactive compounds peak area, height,

peak retention time and mass spectral fragmentation patterns in relation with 62,000 known components stored in National Institute of Standards and Technology library was described. The name, molecular weight and structure of the compound were ascertained.

Statistical analysis

The statistical differences were calculated using one-way ANOVA followed by Dunnet test (Control Vs test). $P<0.05$ was considered to be significant. Results were expressed as mean ± standard deviation.

RESULTS

Qualitative analysis of phytochemicals

The decoction extract of *G. globosa* revealed the presence of alkaloid, flavonoid, anthocyanins, coumarins, proteins and amino acids. Hydroalcoholic extract showed the presence of alkaloid, flavonoid, carbohydrates, tannins, anthocyanin, protein and amino acids (Table 1).

Assay of anti-inflammatory activity under *in vitro* condition

Inhibition of albumin denaturation assay

The evaluation of *G. globosa* flower extracts for its anti-inflammatory activity against denaturation of egg albumin is represented in Figure 2. All extracts and diclofenac sodium (standard) showed concentration dependent protein inhibition that ranges from 100µg to 500µg/mL. The decoction extract showed 87.71% of inhibition ($p \leq 0.05$), followed by hydroalcoholic extract that showed 80.70% at 500g/mL, whereas diclofenac sodium was found to be 91.22% at 500g/mL ($p \leq 0.05$).

Antiproteinase action assay

The significant antiproteinase activity of *G. globosa* was observed at different concentrations ranging from 100µg to 500µg/mL shown in Figure 3. The maximum inhibition was found to be at 500µg/mL for decoction extract ($p \leq 0.05$) followed by hydroalcoholic extract with 87.71% of inhibition ($p \leq 0.05$).

Hypotonicity induced haemolysis

The effect of *G. globosa* flower extracts on hypotonicity induced hemolysis was summarized in Figure 4. The percentage protection was found to be 82.45% ($p \leq 0.05$) for decoction extract followed by hydroalcoholic extract of 68.75% at 500µg/mL. The standard offered 75% ($p \leq 0.05$) of protection against the lysing effect of hypotonic solution.

Inhibition of hemolysis by *G. globosa* extracts

The *G. globosa* extract significantly inhibited the heat induced hemolysis at a concentration ranging from 100µg to 500µg/mL (Figure 5). The significant membrane protection against lysis induced by heat was found to be at 500µg/mL. The standard aspirin showed 78.94% followed by decoction extract and hydroalcoholic extract with 68.42% and 63.12% respectively ($p \leq 0.05$).

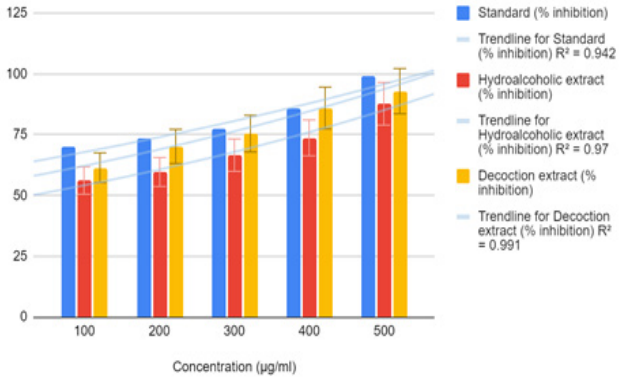

Figure 3: Anti-proteinase action assay of *G. globosa* extracts (Values are expressed as mean±SD and $p<0.05$ vs control).

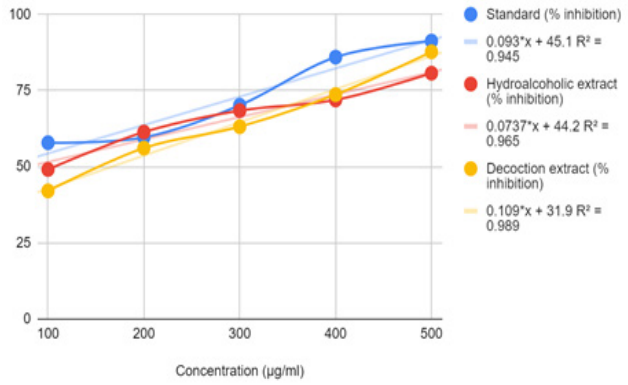

Figure 2: Inhibition of albumin denaturation assay by *G. globosa* extracts (Values are expressed as mean±SD and $p<0.05$ vs control).

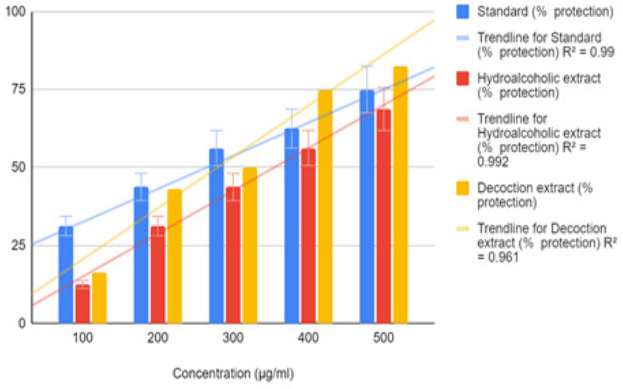

Figure 4: Hypotonicity induced hemolysis by *G. globosa* extracts (Values are expressed as mean±SD and $p<0.05$ vs control).

Estimation of total phenolic content

The total phenolic content of *G. globosa* flower extacts was shown in Table 2. The calibration curve of gallic acid was used to calculate the total phenolic content Figure 6. The phenol content was expressed as mcg/mL. In decoction extract total phenolic content was found to be 60mcg/mL followed by hydroalcoholic extract (45mcg/mL).

DPPH-free radical scavenging assay

The flower extracts showed scavenging capacity in a concentration dependent manner (Figure 7). When compared with standard, the decoction extract showed 79.16% of reducing capacity followed by hydroalcoholic extracts 40.27% at 500µg/mL ($p \leq 0.05$).

GC-MS analysis

GC-MS analysis of hydroalcoholic flower extract of *G. globosa* was performed using SHIMADZU QP2020 and the compounds was found to be polar in nature. The molecular formula, molecular weight, name of the compound and retention time was depicted in Figure 8 and Table 3. Out of 29 compounds, the major identified compounds were Benzene 1,3, di chloro (R_T 5.67), 1, 2-propanediol, 1-phenyl-1, 2-propanediol (R_T 5.95), Cyclotrisiloxane, hexamethyl- (6.27), Cyclohexane,1-bromo-1-(ethoxymethoxymethyl)-(1-bromocyclohexane) carboxy aldehyde ethyl met (R_T 8.51), Tetracosane (R_T 14.78), trichlorodecyl-,1,2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (R_T 22.52), and Ethyl 2-[5-phenyl-5-[trimethylsilyl)oxy] pentyl]acrylate (R_T 20.94).

DISCUSSION

The present study was carried out to evaluate the anti-inflammatory activity of decoction and hydroalcoholic extract of *G. globosa*. In all the tests carried out, the decocotion extract showed potent results when compared with hydroalcoholic extract. Inflammation is a biological protective process that helps in tissue healing. Several chemical mediators such as prostaglandins, leukotrienes, prostacyclin's, lymphokines and chemokines are produced through several pathways to induce immune response against external stimuli.[21,22] In recent times, several plant-based compounds play an important role to replace anti-inflammatory drugs of synthetic origin.[23,24] Each plant possesses unique anti-inflammatory inhibition mechanisms such as inhibition of 15-lipoxygenases,

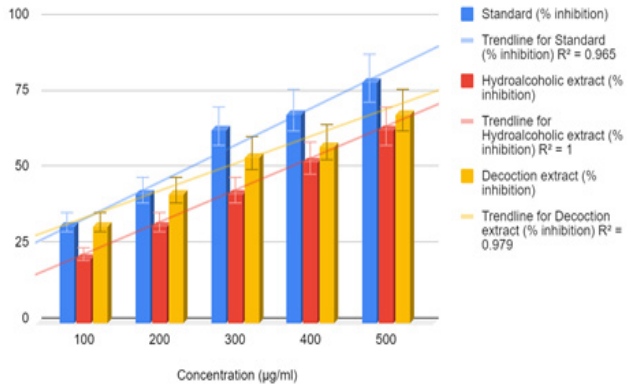

Figure 5: Inhibition of hemolysis by *G. globosa* extracts (Values are expressed as mean±SD and $p<0.05$ vs control).

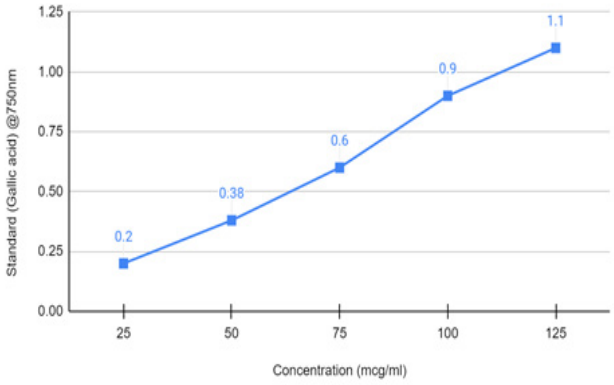

Figure 6: Calibration curve of Gallic acid.

Table 1: Phytochemical screening of *G. globosa* flower extracts.

S. No	Phytoconstituents	Decoction extract	Hydroalcoholic extract
1	Alkaloid	Positive	Positive
2	Flavonoid	Positive	Positive
3	Carbohydrate	Negative	Positive
4	Proteins and aminoacids	Positive	Positive
5	Anthocyanin	Positive	Positive
6	Coumarins	Positive	Negative
7	Tannin	Negative	Positive

nitric oxide synthase, cyclooxygenase, phospholipase A2, proinflammatory cytokines and modulating pro-inflammatory gene expression.[24-26] The use of plant extracts in inflammation has been used since decades and those bioactive compounds can either act individually or synergicaly to exert their actions. In inflammation and arthritic disease, denaturation of proteins plays an important role. Drugs that prevent protein denaturation can be helpful in anti-inflammatory drug production.[27] Several thousands of scientific papers have been emerging to focus on herbal extracts and its bioactive compounds especially alkaloids, terpenes and phenolic compounds are gaining importance in recent times.[28] The secondary metabolites from plants are known to

Table 2: Total phenolic content of *G. globosa* extracts.

S.No	Extract	Absorbance 750nm	Phenolic content mcg/ml
1.	Hydroalcoholic extract	0.36±0.01	45
2.	Decoction extract	0.47±0.01	60

Table 3: Phytoconstituents in hydroalcoholic flower extract of *G. globosa*.

S.No	R_T	Name of the compound	Molecular formula	Molecular weight
1	5.67	Benzene 1,3, di chloro	$C_6H_4C_{12}$	146
2	5.950	1, 2-propanediol, 1-phenyl	$C_9H_{12}O_2$	152
3	6.275	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222
4	6.740	Benzene, nitro-	$C_6H_5NO_2$	123
5	8.515	Cyclohexane,1-bromo-1-(ethoxy methoxy methyl)- (1-bromo cyclo hexane) carboxy aldehyde ethyl met	$C_{10}H_{19}B_rO_2$	250
6	8.515	1,3,5-trioxane	$C_3H_6O_3$	90
7	12.265	Hexane,3,3-dimethyl- 3,3-dimethyl hexane	C_8H_{18}	114
8	12.485	Phenol,2,4-bis(1,1-dimethylethyl)- 2,4-ditert-butylphenol 1-hydroxy-2,4-di-tert-butyl benzene	$C_{14}H_{22}O$	206
9	14.780	Tetracosane	$C_{24}H_{50}$	338
10	14.830	Methyl -2,3,4,5-tetrahydro-4-methyl-1,5-dioxo-1h-benz[c]azepin-3-carboxylate	$C_{13}H_{13}NO_4$	247
11	14.380	1,7-dioxaspiro [5,5] undecane-4,5-diol,2-ethyl-3-methyl-10-(phenyl methoxy)-8-(phenyl methoxy) methyl-,5	$C_9H_{38}O_7$	498
12	15.265	1-iodotetra decane einecs-3 myristic iodide tetradecane, i-iodo- tetradecyl iodide	$C_{14}H_{29}I$	324
13	15.265	Octadecane	$C_{18}H_{38}$	254
14	15.405	Tetra decanoic acid	$C_{14}H_{28}O_2$	228
15	16.995	Silane, trichloro decyl-	$C_{10}H_{21}C_{13}Si$	276
16	17.145	Tetra decanoic acid ,12-methyl-methyl ester methyl 12-	$C_{16}H_{32}O_2$	256
17	17.145	Nonanoic acid,7-methyl-methyl ester	$C_{11}H_{22}O_2$	186
18	17.145	Methyl 6-hydroxy caproate hexanoic acid	$C_7H_{14}O_3$	146
19	17.145	Methyl decanoate	$C_{23}H_{46}O_2$	354
20	17.220	Benzene propanoic acid,3,5-bis(1,1-dimethylethyl)-4-hydroxy-methyl ester	$C_{18}H_{28}O_3$	292
21	17.375	5,10-diethoxy-2,3,7,8-tetrahydro-1h,6h-dipyrrolo[1,2-a:1,2-d] pyrazine 5,10-diethoxy-2,3,7,8-tetrahydro-1h	$C_{14}H_{22}N_2O_2$	250
22	19.880	1-Dodecanamine, N-methyl-N-nitroso	$C_{13}H_{28}N_2O$	228
23	20.940	Ethyl 2-[5-phenyl-5-[trimethylsilyl)oxy] pentyl] acrylate	$C_{19}H_{30}O_3Si$	334
24	20.940	3-(2,4-dimethoxy-5-pyrimidinyl)-2-propene-1-sulfonamide	$C_9H_{13}N_3O_4S$	259
25	22.180	Phosphoro chloridic acid, nonyl pentyl ester	$C_{14}H_{30}ClO_3P$	312
26	22.350	Hexadecenoic acid,2-hydroxy-1-(hydroxyl methy)ethyl ester	$C_{19}H_{38}O_4$	330
27	22.350	Octadecanoic acid,2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358
28	22.520	1,2-benzene dicarboxylic acid, 1,2-benzene dicarboxylic acid,bis (2-ethyl hexyl) ester 1,2-benzenedic	$C_{24}H_{38}O_4$	390
29	22.520	Phthalic acid, heptadecyltrans-hex-3-enyl ester	$C_{13}H_{50}O_4$	486

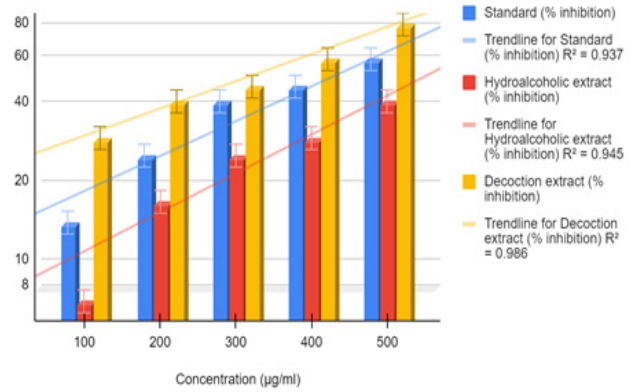

Figure 7: DPPH Scavenging assay of *G. globosa* extracts (Values are expressed as mean±SD and $p<0.05$ vs control).

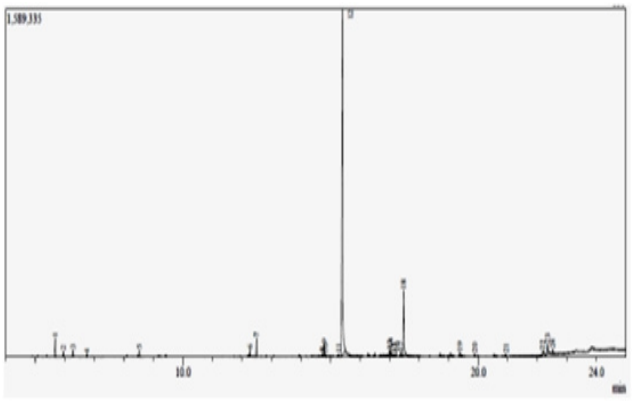

Figure 8: GC-MS chromatogram of hydroalcoholic flower extract of *G. globosa*.

possess anti-microbial, anti-inflammatory, anti-cancer, anti-proliferative and so on.[29] The present study reveals the presence of alkaloid, flavonoid, carbohydrates, anthocyanins, coumarins, tannin's, proteins and amino acids in hydroalcoholic and decoction extract respectively and these results are in line with.[30-32] This result showed that phenols, flavonoids and alkaloids are potential phytoconstituents that might have some pharmacological activities. It was reported that flavonoids can inhibit prostaglandin synthesis through COX and lipoxygenase pathway.[28]

The erythrocyte membrane stabilization used in this study is analogous to lysosomal membrane. Lysosomal stabilization will prevent the release of activated neutrophils, enzymes and protease that leads to further inflammation.[33] Hence to assume, the stabilization of *G. globosa* flowers extracts in this study may also stabilize the lysosomal membrane there by inhibiting the release of neutrophils, proteinase which in turn cause further damages to tissue inflammation. In previous studies anti-inflammatory and antioxidant activity has been reported in bioactive compounds such as flavanoids, tannins and phenol that act either individually or by synergistic mechanism. Naturally phenolic compounds inhibit the action or production of proinflammatory mediators.[33] In this study an attempt was made to assess the concentration of phenol in *G. globosa* extracts. The highest concentration of phenol was observed in decoction extract with 60mcg/mL.

During inflammation, the concentration of reactive oxygen species will be high that leads to imbalance between oxidizing molecules and antioxidant system.[34] From the results of DPPH assay, the decoction extract of *G. globosa* showed maximum potent scavenging activity (79.16%) and hydroalcoholic extract showed least activity (40.27%), this was in accordance with.[28] The arrangement and number of hydroxyl groups in phenolic ring of polyphenols determines the antioxidant activity.[34] Flavonoids play an essential role in preventing ROS formation by inhibiting enzymes and thereby providing antioxidant defense mechanism. Next to flavonoids, polyphenols[26] and tannins are also reported to have antioxidant activity.[35]

GC-MS is a versatile technique for identification and quantification of unknown organic compounds by detecting the relationship between phytoconstituents and pharmacology.[36] In GC-MS analysis of hydroalcoholic flower extract of *G. globosa*, the compound 1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester (R_T 22.50) was reported to possess antioxidant and anti-inflammatory activity,[37] fluoro uridine (R_T 22.18) possess antimicrobial activity,[38] Tetradecanoic acid (R_T 15.40) known to have larvicidal and repellent activity, silane as coupling agent[39] (R_T 16.99), Tetracosane (R_T 14.7) possess cytotoxicity against MDA-MB-231, AGS cell, NIH 3T3 cells, HT 29[40,41] and cyclotrisiloxane, hexamethyl- (R_T 6.27) possess antibacterial activity.[42,43]

CONCLUSION

Finally, to conclude the results of this work indicates that decoction flower extract of *G. globosa* revealed potent anti-inflammatory activity due to the presence of alkaloid, flavanoid, anthocyanin, coumarins, proteins and aminoacid. The extract also possesses reducing power ability and inhibited albumin denaturation, stabilized red blood cell membrane and anti-proteinase action. The hydroalcoholic extract showed moderate anti-inflammatory activity. The activities were related to dose and the result was in line with the traditional use of the plant in inflammatory conditions. The isolation of individual alkaloids, flavonoids, coumarins, anthocyanin, phenolic compounds and tannins are necessary in this fraction and its effects on several *in vivo* studies are necessary to examine their role in drug development.

Further, the lead compound from *G. globosa* can be used as a potent anti-inflammatory drug for treatment of various diseases.

ACKNOWLEDGEMENT

I extend my sincere gratitude to PG and Research Department of Biotechnology, Jamal Mohamed College (Autonomous), Trichy, Tamilnadu for providing all the facilities to conduct this work.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

ABBREVIATIONS

COX: Cyclooxygenase; **NSAID:** Non-Steroidal Anti-Inflammatory Drug; **RT:** Retension Time.

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Cite this article: Tarnam AY. *In vitro* Evaluation of Purple Inflorescence of *Gomphrena globosa* (L.) Extracts for Anti-inflammatory Activity and its GC/MS Profile. Asian J Biol Life Sci. 2021;10(1):123-31.