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# Chemical and biological investigations of the leaves of *Gynura procumbens*

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**Key words:** *Gynura procumbens*, antimicrobial activity, cytotoxicity, antioxidant property, stigmasterol.

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**Abstract**

The crude *n*-hexane (HX), dichloromethane (DCM), methanol (ME) and ethyl acetate (EA) extracts of the leaves of *Gynura procumbens* were subjected to antioxidant, antibacterial and antifungal activities and cytotoxicity against brine shrimp nauplii. The DCM and EA extracts exhibited mild antimicrobial activities, whereas, HX and ME extracts did not show any sort of sensitivity. On the other hand, the brine shrimp lethality with $LC_{50}$ values was 70.71, 0.78, 4.42 and 59.46 µg/mL for HX, DCM, ME and EA extracts, respectively indicates the presence of potent bioactive compounds. At the same time, among the four crude extracts, only ME and HX extracts showed potential antioxidant activity with $IC_{50}$ values of 20.35 and 48.0µg/mL. A pure compound was isolated from HX extract and the structure of the compound was elucidated as stigmasterol by means of $^1H$ NMR spectroscopy.

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## Introduction

*Gynura procumbens* is belonging to the genus *Gynura*, family Asteraceae. It is known for a wide variety of phytochemicals and pharmacological properties. This herb has been used as traditional medicine for household remedy against various human ailments such as eruptive fevers, rash, kidney disease, migraines, constipation, hypertension, diabetes mellitus and cancer (Perry, 1980).

*G. procumbens* plant is an annual evergreen shrub and usually grows up to a height of 1-3 m, with a fleshy stem and purple tint. The leaves are ovate-elliptic or lanceolate, 3.5 to 8 cm long and 0.8 to 3.5 cm wide. Flowering heads are panicled, narrow, yellow and 1 to 1.5 cm long. *G. procumbens* is found in Southeast Asia, particularly in Indonesia, Malaysia, the Philippines and Thailand. However, this plant is not native to Bangladesh. It was collected by the corresponding author (Dr. Rahman) of this article from an NGO worker. In 2007, when that NGO worker came to know that the father of Dr. Rahman is a diabetic patient he brought this plant from Malaysia to Bangladesh as he (NGO worker) heard that this plant is widely used as an accepted medicine for diabetes.

The literature survey reveals that leaves or leaves extracts of *G. procumbens* has anti-herpes simplex virus (Nawawi *et al.*, 1999), antihyperglycemic (Li *et al.*, 2009; Akowuah *et al.*, 2002), antihyperglycaemic and antihyperlipidaemic (Zhang and Tan, 2000), anti-inflammatory (Iskander *et al.*, 2002), anticarcinogenic (Agustina *et al.*, 2006), blood hypertension reduction capabilities (Hoe *et al.*, 2006; Kim *et al.*, 2006), antiproliferative on human mesangial cell (Lee *et al.*, 2007), antioxidative (Puangpronpitag *et al.*, 2010; Rosidah *et al.*, 2008) and anti-ulcerogenic (Mahmood *et al.*, 2010) properties. The leaves of this plant are often consumed in diet and research shows that leaves contents are not having any toxic effects (Rosidah *et al.*, 2009). The benefits of the traditional use of *G. procumbens* have also been supported by the isolation and identification of several possible flavonoid, saponin, tannin and terpenoid constituents from this plant (Akowuah *et al.*, 2002). *G. procumbens* is an important tropical medicinal plant that has been studied mainly in Southeast Asian countries for its medicinal properties. To the best of our knowledge, no research work had been conducted yet in Bangladesh with this plant. The current study deals for the first time with Bangladesh originated *G. procumbens* to know its chemical and biological properties. Here, we report on antibacterial, antifungal and antioxidant activities of the leaf extracts of *Gynura procumbens*, and have included the test for cytotoxicity using brine shrimps. Furthermore, an attempt has been taken to isolate the components from different extracts and we also report here the isolation of a pure compound from *n*-hexane extract.

## Materials and methods

### *Plant materials*

The leaves of *G. procumbens* were collected from the botanical garden of Curzon Hall campus of Dhaka University, Bangladesh and the plant was identified by prominent botanist Prof. Dr. Md. Abul Hasan, former Chairman of the Department of Botany, University of Dhaka. The leaves were thoroughly washed with clean water to remove earthy matters. The leaves were sun-dried for several days and then oven-dried for 24 hrs at 40 °C for better grinding. The dried leaves were then ground into coarse powder.

### *Extraction*

About 200 gm of the coarse powder of the leaves of Gynura procumbens was successively extracted with n-hexane (HX), dichloromethane (DCM), methanol (ME) and ethyl acetate (EA) of increasing polarity for one week at room temperature with occasional shaking. The individual extracts were then filtered off through a cotton plug followed by Whatman No. 1 filter paper. The volume of each filtrate was reduced using Buchii rotary evaporator at low temperature and pressure, which afforded n-hexane (2.14 gm), dichloromethane (1.59 gm), methanol (2.05 gm) and ethyl acetate (2.58 gm) soluble materials.

*Chemical investigation of crude extracts*

The concentrated crude *n*-hexane extract (2.14 gm) was subjected to column chromatography for fractionation on silica gel (Kieselgel 60, mesh 70-230) and eluted with 5:1 hexane/ethyl acetate solvent system to afford 32 fractions (each 100 ml). When fractions 14-16 were combined together in a beaker and after one day, when the solvent was air evaporated, some fine colorless crystals were obtained at the bottom of the beaker with some impurities. The bulk was then subjected to solvent treatment. Actually, it was washed with *n*-hexane in a sample vial and after repeated washing 20mg of colorless needle-shaped crystals (GP) was obtained. When the sample was eluted with toluene: ethyl acetate (95:5) on a silica gel TLC plate, a single spot with a $R_f$ value of 0.72 was observed under UV lamp at 254 nm and showed purple color after spraying with vanillin-sulfuric acid followed by heating. On the other hand, thin layer chromatography (TLC) screening of crude dichloromethane, crude methanol and crude ethyl acetate extracts showed tailing with different solvent systems and seemed difficult to isolate the components from each extract. Thus, phytochemical investigations of these three crude extracts were avoided this time but investigations for antimicrobial, cytotoxicity and antioxidant properties were conducted for each extract.

*Microorganisms*

Thirteen bacteria (5 Gram positive and 8 Gram negative) and three fungi, collected from the stock cultures of the Institute of Nutrition and Food Science, University of Dhaka, were used for the antimicrobial assays.

*Antimicrobial tests*

Antibacterial and antifungal activities were tested by the disc-diffusion method (Bauer, *et al.,* 1966). The crude extracts (HX, DCM, ME and EA) and the pure compound (GP) were dissolved separately in methanol and applied to sterile filter paper discs at 400µg/disc and carefully dried to evaporate the residual solvent. Discs containing the test materials were then placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard disc of kanamycin (30µg/disc) and blank discs (impregnated with methanol followed by evaporation) were used as positive and negative controls, respectively. These plates were then kept at low temperature (4 °C) for 24 hours to allow maximum diffusion of test samples. The plates were then incubated at 37 °C for 24 hours to allow maximum growth of the organisms. The test materials having antimicrobial activity inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter. The experiment was carried out in triplicate and the average zone of inhibition was calculated.

*Brine shrimp lethality test*

Brine shrimp lethality bioassay technique of Meyer (Meyer *et al.,* 1982) was applied for the determination of cytotoxic property of the leaf extracts of *Gynura procumbens*. The crude *n*-hexane (HX), dichloromethane (DCM), methanol (ME) and ethyl acetate (EA) extracts were separately dissolved in DMSO. Four mg of each of the crude extracts (HX, DCM, ME and EA) was dissolved in DMSO and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563, 0.78125 µg/mL were obtained by serial dilution technique. Vincristine sulphate (VS) and DMSO were used as the positive and negative control, respectively. Then the solutions were added to the premarked vials containing ten live brine shrimp nauplii in 5 mL simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From the data, the percent (%) of lethality of the brine shrimp was calculated for each concentration. The median lethal concentration ($LC_{50}$) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration.

*Antioxidant activity test*

The free radical scavenging activity (antioxidant potential) of different crude extracts (HX, DCM, ME and EA) of the leaves of *G. procumbens* on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated by the method of Brand-Williams (Brand-Williams *et al.*, 1995). Two mL of methanol solution of the crude extracts (2 mg) at different concentrations such as 500, 250, 125, 62.5, 31.25, 15.625, 7,831, 3.906, 1.953 and 0.977 µg/mL were mixed with 3 mL methanolic solution of DPPH (20µg/mL). After 30 min reaction period at room temperature in dark place, absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by plant extracts as compared to that produced by the standard antioxidant agents of *tert*-butyl-1-hydroxytoluene (TBHT) and ascorbic acid (ASA). Extract concentration providing 50% inhibition ($IC_{50}$) was calculated from the graph obtained by plotting inhibition percentage against extract concentration.

**Results and discussion**

*Antimicrobial activity*

The result of the antimicrobial activities of crude *n*-hexane (HX), dichloromethane (DCM), methanol (ME) and ethyl acetate (EA) extracts of *G. procumbens* and pure Compound (GP) has been summarized in Table 1. Present investigation showed that only DCM and EA extracts have the mild sensitivity against almost all the bacteria and fungi (except *Staphylococcus aureus* and *Escherichia coli*), whereas crude HX and EA extracts and compound GP did not show any antimicrobial activity. The average zones of inhibition produced by DCM and EA extracts were found to be 6-7mm at a concentration of 400µg/disc.

**Table 1**. Antimicrobial activity of the leaf extracts of *Gynura procumbens*.

| Test Microorganisms | Diameter of zone of inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | HX | DCM | ME | EA | GP | Kanamycin |
| **Gram positive bacteria** | | | | | | |
| *Bacillus cereus* | - | 7 ± 0.10 | - | 7 ± 0.09 | - | 35 ± 0.25 |
| *Bacillus megaterium* | - | 7 ± 0.40 | - | 7 ± 0.15 | - | 35 ± 0.40 |
| *Bacillus subtilis* | - | 7 ± 0.12 | - | 7 ± 0.19 | - | 35 ± 0.20 |
| *Staphylococcus aureus* | - | - | - | - | - | 36 ± 0.36 |
| *Sarcina lutea* | - | 7 ± 0.32 | - | 7 ± 0.11 | - | 37 ± 0.21 |
| **Gram negative bacteria** | | | | | | |
| *Escherichia coli* | - | - | - | - | - | 37 ± 0.40 |
| *Pseudomonas aeruginosa* | - | 6 ± 0.16 | - | 7 ± 0.17 | - | 37 ± 0.16 |
| *Salmonella paratyphi* | - | 7 ± 0.25 | - | 7 ± 0.09 | - | 37 ± 0.26 |
| *Salmonella typhi* | - | 7 ± 0.10 | - | 7 ± 0.17 | - | 35 ± 0.15 |
| *Shigella boydii* | - | 6 ± 0.25 | - | 6 ± 0.10 | - | 37 ± 0.12 |
| *Shigella dysenteriae* | - | 6 ± 0.13 | - | 7 ± 0.12 | - | 37 ± 0.21 |
| *Vibrio mimicus* | - | 7 ± 0.15 | - | 7 ± 0.16 | - | 37 ± 0.22 |
| *Vibrio parahemolyticus* | - | 7 ± 0.12 | - | 7 ± 0.16 | - | 38 ± 0.42 |
| **Fungi** | | | | | | |
| *Candida albicans* | - | 6 ± 0.15 | - | 7 ± 0.16 | - | 37 ± 0.10 |
| *Aspergillus Niger* | - | 7 ± 0.18 | - | 7 ± 0.19 | - | 37 ± 0.23 |
| *Sacharomyces cerevacae* | - | 6 ± 0.15 | - | 6 ± 0.18 | - | 38 ± 0.29 |

The diameter of zone of inhibition is expressed as mean ± SD (n=3); a diameter less than 6 mm was considered as inactive; HX: crude *n*-hexane extract; DCM: crude dichloromethane extract; ME: crude methanol extract; EA: crude ethyl acetate extract; GP: isolated pure compound; KAN: Kanamycin; "-" indicates no activity.

Therefore, it can be concluded from the above study that the DCM and EA extracts of *G. procumbens* contain some antimicrobial components.

*Brine shrimp lethality*

It was found from the result of the brine shrimp lethality test (Table 2) that the crude extracts (HX, DCM, ME and EA) of *G. procumbens* exhibited toxicity towards brine shrimp. Test samples showed different mortality rate at different concentrations. The mortality rate of brine shrimp was found to be increased with the increase of the concentration for each sample. The percent mortality of the brine shrimp nauplii was calculated for every concentration for each sample. A plot of log concentration of the sample versus percent of mortality showed an approximate linear correlation between them. The positive control groups showed non linear mortality rates at lower concentrations and linear rates at higher concentrations. There was no mortality in the negative control groups indicating the test as a valid one and the results obtained are only due to the activity of the test samples. $LC_{50}$ obtained from the best-fit line slope were 0.44, 70.71, 0.78, 4.42 and 59.46 µg/mL for VS (Std.), HX, DCM, ME and EA extracts respectively. In comparison to positive control (vincristine sulphate), the cytotoxicity exhibited by DCM extract of *G. procumbens* was promising and this clearly indicates the presence of potent bioactive compounds. On the other hand, this study also revealed that the ME extract has moderate cytotoxicity, whereas HX and EA extracts have mild cytotoxicity.

**Table 2.** Brine shrimp lethality of the crude extracts of *Gynura procumbens*.

| Sample | $LC_{50}$ (µg/mL) | Regression equation | $R^2$ |
|---|---|---|---|
| VS | 0.44 ± 0.01 | y = 0.5805x + 1.502 | 0.7947 |
| HX | 70.71 ± 1.33 | y = 0.5841x + 0.611 | 0.7222 |
| DCM | 0.78 ± 0.23 | y = 0.2818x + 4.534 | 0.8744 |
| ME | 4.42 ± 0.54 | y = 0.3955x + 3.636 | 0.9511 |
| EA | 59.98 ± 1.77 | y = 0.7503x - 1.237 | 0.8154 |

The values of $LC_{50}$ are expressed as mean ± SD (n=3). VS: vincristine sulphate (Std.); HX: crude *n*-hexane extract; DCM: crude dichloromethane extract; ME: crude methanol extract; EA: crude ethyl acetate extract.

**Table 3.** $IC_{50}$ values of standard and test samples of *Gynura procumbens*.

| Sample | $IC_{50}$ (µg/mL) |
|---|---|
| TBHT (Standard) | 27.5 ± 0.54 |
| ASA | 5.8 ± 0.21 |
| HX | 48.0 ± 0.96 |
| DCM | 355.0 ± 3.35 |
| ME | 20.35 ± 1.47 |
| EA | 221.0 ± 1.67 |

The values of $IC_{50}$ are expressed as mean ± SD (n=3). TBHT: *tert*-butyl-1-hydroxytoluene; ASA: ascorbic acid; HX: crude *n*-hexane extract; DCM: crude dichloromethane extract; ME: crude methanol extract; EA: crude ethyl acetate extract.

*Antioxidant potential*

Different crude extracts (HX, DCM, ME and EA) of *G. procumbens* were subjected to free radical scavenging activity to evaluate the antioxidant potential of *Gynura procumbens*, where *tert*-butyl-1-hydroxytoluene (TBHT) and ascorbic acid (ASA) was used as reference standard. The results are summarized in Table 3. It has been observed that ME extract showed the highest antioxidant activity with $IC_{50}$ value of 20.35µg/mL, which is comparable to that of the reference standard TBHT (27.5µg/mL) and it is clear that ME extract might play an important role in preventing free radical induced-diseases. At the same time, HX extract also showed potent antioxidant activity (48.0µg/mL). On the other hand, DCM and EA extracts showed only mild antioxidant activity, whose $IC_{50}$ values were 355.0µg/mL and 221.0µg/mL, respectively.

*Characterization of the isolated compound*

The structure of the isolated compound (GP) was determined by $^1$H NMR analysis as well as by comparing with the previously reported values. The $^1$H NMR spectrum (400MHz, $CDCl_3$) of GP displayed a multiplet for one proton at $\delta$ 3.51, indicative of H-3 of the steroidal nucleus and a broad singlet at $\delta$ 3.72 indicated the presence of –OH group at C-3. The typical signal for H-6 of the steroidal skeleton was evident from a multiplet at $\delta$ 5.33 that integrated for one proton. The olefinic protons H-22 and H-23 appeared as characteristic downfield signals at $\delta$ 5.14 and $\delta$ 5.03, respectively with a coupling constant of 15.0, which is indicative for *trans*-coupling with the olefinic proton and vicinal coupling with neighboring methene proton. Two tertiary methyl groups at $\delta$ 0.68 and $\delta$ 1.00 are assignable for C-13 and C-10, respectively. In addition, two doublets centered at $\delta$ 0.83 could be ascribed to the methyl groups at C-25 and another doublet at $\delta$ 0.92 integrating for three protons was demonstrative of a methyl group at C-20. The triplet at $\delta$ 0.81 could be demonstrated to the methyl group at C-28. All these spectral features of the compound GP were found to be compatible with the structure of stigmasterol (Khan, 1991) and the structure of GP (stigmasterol) has been represented in Fig. 1.

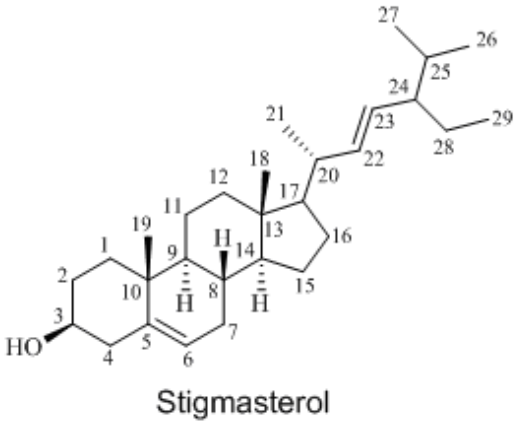


**Fig. 1.** Chemical structure of isolated compound (GP).

*Stigmasterol*

Colorless needles; mp 160-163 °C; $^1$H NMR ($CDCl_3$) $\delta$ 0.66 (3H, s, position-18), 0.80 (3H, t, position-29), 0.80 (3H, d, *J* 6.0 Hz, position-26), 0.84 (3H, d, *J* 6.0 Hz, position-27), 0.91 (3H, d, *J* 6.5 Hz, position-21), 1.02 (3H, s, position-19), 3.51 (1H, m, position-3), 3.72 (1H, s, –OH, position-3), 5.03 (1H, dd, *J* 15.0 Hz, *J* 8.3 Hz, position-23), 5.14 (1H, dd, *J* 15.0 Hz, *J* 8.3 Hz, position-22), 5.33 (1H, m, position-6).

**Conclusion**

It has been found from the above study that the different crude extracts of *G. procumbens* have antimicrobial and cytotoxic activities, which supports the traditional uses of this herb for the treatment of bacterial and fungal infections. The high lethality to brine shrimp nauplii indicates that this plant might contain antitumour or pesticidal compounds. Potent antioxidant property of crude methanol (ME) extract of this plant will explore its significant utility in reducing the diseases or disorders caused by oxidative stresses. In the present study, we have isolated stigmasterol and detailed chemical investigation is further required to isolate the molecules that are responsible for antimicrobial and antioxidant activities.

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**References**

**Perry LM.** 1980. Medicinal Plants of East and Southeast Asia: Attributed Properties and Uses. 1st Edn., The MIT Press, Cambridge, Massachusetts and London, ISBN: 0-262-16076-5, 334- 360.

**Nawawi A, Nakamura N, Hattori M, Kurokawa M, Shiraki K.** 1999. Inhibitory effects of Indonesian medicinal plants on the infection of herpes simplex virus type 1. Phytotherapy Research **13,** 37- 41. http://dx.doi.org/10.1002/(SICI)1099-1573(199902)13:1<37::AID-PTR382>3.0.CO;2-S/pdf

**Li WL, Ren BR, Zhuo M, Hu Y, Lu CG.** 2009. The anti-hyperglycemic effect of plants in genus *gynura* cass. The American Journal of Chinese Medicine **37,** 961- 966. http://www.ncbi.nlm.nih.gov/pubmed/19885955

**Akowuah GA, Sadikun A, Mariam A.** 2002. Flavonoid identification and hypoglycemic studies of butanol fraction from *Gynura procumbens*. Pharmaceutical Biology **40,** 405- 410. http://dx.doi.org/10.1076/phbi.40.6.405.8440

**Zhang XF, Tan BKH.** 2000. Effects of an ethanolic extract of *Gynura procumbens* on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. Singapore Medical Journal **41,** 9- 13. http://cat.inist.fr/?aModele=afficheN&cpsidt=269964

**Iskander MN, Song Y, Coupar IM, Jiratchariyakul W.** 2002. Antiinflammatory screening of the medicinal plant *Gynura procumbens*. Plant Foods for Human Nutrition **57,** 233- 244. http://dx.doi.org/10.1023/A:1021851230890

**Agustina D, Haryana SM, Supartinah A.** 2006. Anticarcinogenesis effect of *Gynura procumbens* (Lour) merr on tongue carcinogenesis in 4NQO-induced rat. Dental Journal (Maj. Ked. Gigi) **39,** 126- 132.

**Hoe SZ, Kamaruddin MY, Lam SK.** 2006. Inhibition of angiotensin-converting enzyme activity by a partially purified fraction of *Gynura procumbens* in spontaneously hypertensive rats. Medical Principles and Practice **16,** 203- 208. http://dx.doi.org/10.1159/000100391

**Kim MJ, Lee HJ, Wiryowidagdo S, Kim HK.** 2006. Antihypertensive effects of *Gynura procumbens* extract in spontaneously hypertensive rats. Journal of Medicinal Food **9,** 587- 590. http://dx.doi.org/10.1089/jmf.2006.9.587

**Lee HJ, Lee B, Chung J, Wiryowidagdo S, Chun W, Kim S, Kim S, Choe M.** 2007. Inhibitory effects of an aqueous extract of gynura procumbens on human mesangial cell proliferation. Korean Journal of Physiology & Pharmacology **11,** 145- 148.

**Puangpronpitag D, Chaichanadee S, Naowaratwattana W, Sittiwet C, Thammasarn K, Luerang A, Kaewseejan N.** 2010. Evaluation of nutritional value and antioxidative properties of the medicinal plant *Gynura procumbens* extract. Asian Journal of Plant Sciences **9,** 146- 151. http://dx.doi.org/10.3923/ajps.2010.146.151

**Rosidah MFY, Sadikun A, Asmawi M.** 2008. Antioxidant potential of *Gynura procumbens*. Pharmaceutical Biology **46,** 616- 625.

http://dx.doi.org/10.1080/13880200802179642

**Mahmood AA, Mariod AA, Al-Bayaty F, Abdel-Wahab SI.** 2010. Anti-ulcerogenic activity of *Gynura procumbens* leaf extract against experimentally-induced gastric lesionsin rats. Journal of Medicinal Plant Research **4,** 685- 691. http://indianmedicine.eldoc.ub.rug.nl/root/M3/161m/

**Rosidah MFY, Sadikun A, Ahmad M, Akowuah GA, Asmawi MZ.** 2009. Toxicology evaluation of standardized methanol extract of *Gynura procumbens*. Journal of Ethnopharmacology **123,** 244- 249. http://dx.doi.org/10.1016/j.jep.2009.03.011

**Akowuah GA, Sadikun A, Mariam A.** 2002. Flavonoid identification and hypoglycemic studies of butanol fraction from *Gynura procumbens*. Pharmaceutical Biology **40,** 405- 410. http://dx.doi.org/10.1076/phbi.40.6.405.8440

**Bauer AW, Kirby WMM, Sherris JC, Turck M.** 1966. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology **45,** 493- 496.

**Meyer BN, Ferringni NR, Puam JE, Lacobsen LB, Nichols DE, McLaughlin JL.** 1982. Brine shrimp: a convenient general bioassay for active constituents. Planta Medica **45**, 31- 34. http://dx.doi.org/10.1055/s-2007-971236

**Brand-Williams W, Cuvelier ME, Berset C.** 1995. Use of free radical method to evaluate antioxidant activity. Lebensm Wiss Technology **28,** 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5

**Khan RI.** 1991. Natural Product: A Laboratory Guide, 2nd Ed. Academic Press, N.Y, USA.