

- Short Communication

**COMPARATIVE STUDY OF NON POLAR AND POLAR SOLVENT
EXTRACTS OF *GLOCHIDION VELUTINUM* WIGHT IN RESPECT OF
ANTIOXIDANT AND ANTIBACTERIAL ACTION**

MD. SELIM HOSSAIN*, ANANTA KUMAR DAS, FAZLAY RABBI AND
MESBAHUDDIN AHMAD

*Department of Pharmacy, Gono Bishwabidyalay, Mirzanagar, Savar, Dhaka,
Bangladesh*

Antioxidant and antimicrobial activities of non-polar and polar extracts of *Glochidion velutinum* Wight have been studied. The phenolic content was determined by Folin-Ciocalteu reagent and colorimetric method was used to quantify flavonoids. Antioxidant activity was also measured by DPPH free radical scavenging assay and disk diffusion method was applied to test antimicrobial activity. Of the hexane, chloroform, ethanol and methanol extracts of *Glochidion velutinum*, higher total phenolic and flavonoid contents were found in methanol and ethanol extracts. Moreover, ethanol and methanol extracts also showed better antimicrobial and antioxidant activities than the other two extracts. The presence of various phytochemicals e.g. alkaloids, tannins, phenols etc in these extracts also substantiated the observed antioxidant and antimicrobial activities.

Key words: *Glochidion velutinum*, Antioxidant, Antimicrobial, Phytochemicals, Phenolic content, Flavonoid

Plants have been the best source of remedies for curing a variety of diseases and have played key roles worldwide for maintenance of health. Natural products of higher plants are important sources of therapeutic agents. Therefore, many research groups are currently screening various plants for different biological activities (Mothana *et al.* 2010, Mulabagal *et al.* 2007, Leu *et al.* 2006).

Glochidion velutinum Wight (Family-Euphorbiaceae) is a small tree or large shrub upto 9 m with coraceous, pinnate venation leaves, yellow male flowers and globose and depressed fruits branches and leaves. This plant is locally known as matachhar and widely distributed in China, Pakistan, India, Nepal, and Bangladesh (Madhavachetty *et al.* 2008). Several triterpenoids, triterpene glycosides and alkaloids are known to be the constituents of this plant (Sandhya *et al.* 2010). The stem bark of the plant has been reported to be moderately bactericidal (Karuppusamy and Rajasekaran, 2009).

* Corresponding author: <selimmd36@gmail.com>.

Traditionally, this plant is used for treating cancer, diabetes, diarrhoea, inflammation and for the healing of wounds (Madhavachetty *et al.* 2008). Literature survey revealed that leaves of *G. velutinum* are effective against type 2 diabetes (Are *et al.* 2011). In addition, there are also reports in support of antioxidant and cytotoxic potential of this plant (Sandhya *et al.* 2011, Hasan *et al.* 2016). However, no study appears to have been conducted on antioxidant and antimicrobial activities of different extracts of the leaves of this plant, yet. The objective of this work was to compare the antimicrobial and antioxidant properties of four different extracts of the leaves of *G. velutinum*. A great interest in the antioxidant activity of plant extracts exists because of free radicals (e.g., reactive oxygen species) that can be responsible for several diseases, for example, heart disease, stroke, arteriosclerosis and cancer, as well as the aging process (Hoye *et al.* 2008).

The plant *Glochidion velutinum* was identified by the expert of Bangladesh National Herbarium, Mirpur, Dhaka where a voucher specimen bearing the accession number DACB-43211 has been deposited. The leaves of the plant were collected from the forest range of Rajendrapur, Gazipur, Dhaka, Bangladesh during the 2nd week of January 2015. The cleaned leaves were dried in shade for 7 days followed by drying in an oven at 40°C for 2 hours. The leaves were then ground to a coarse powder with the help of a mechanical grinder.

The dried powder (400gm) was taken in four porous bags and placed in the chamber of four soxhlet extractors. The powder was first defatted with n-hexane for 28 hours. The defatted powder was then extracted with chloroform, ethanol and methanol successively as before, each for 28 hours. The extracts thus obtained were separately collected and denoted as hexane extract (HGV), chloroform extract (CGV), methanol extract (MGV) and ethanol extract (EGV).

Preliminary phytochemical screenings of *G. velutinum* extracts were carried out using standard procedures as described by Trease and Evans (1978). All the extracts HGV, CGV, MGV and EGV revealed the presence of flavonoids, carbohydrate and alkaloids. The presence of tannins, steroids and saponins were observed only in CGV, MGV and EGV.

The antioxidant activity was determined by DPPH free radical assay by standard protocol (Braca *et al.* 2001). The scavenging of DPPH free radical is indicated by the deep violet color being turned pale yellow or colorless. Various concentrations (1.56 to 800 µg/mL) of crude extracts were used to assess free radical inhibitory ability and ascorbic acid (1.56 to 50 µg/mL) was taken as standard. The absorbance of crude extracts/ standard was measured at 517 nm with UV-Visible spectrophotometer

(Shimadzu) and free radical inhibitory activity was determined using the following equation:

$$\% \text{ inhibition} = (C-T)/C \times 100$$

where, C = Absorbance of control, T = Absorbance of crude extracts/standard.

Fig. 1 shows the percentage of DPPH free radical inhibition produced by four extracts of *G. velutinum*. There were significant differences in antioxidant activity of HGV ($IC_{50}=30\mu\text{g/ml}$), CGV ($IC_{50}=25.4\mu\text{g/ml}$), MGV ($IC_{50}=0.755\mu\text{g/ml}$) and EGV ($IC_{50}=2.6\mu\text{g/ml}$). Antioxidant activity of MGV was much higher than those of the other extracts. From the results, it is also seen that n-hexane extract possesses less antioxidant activity.

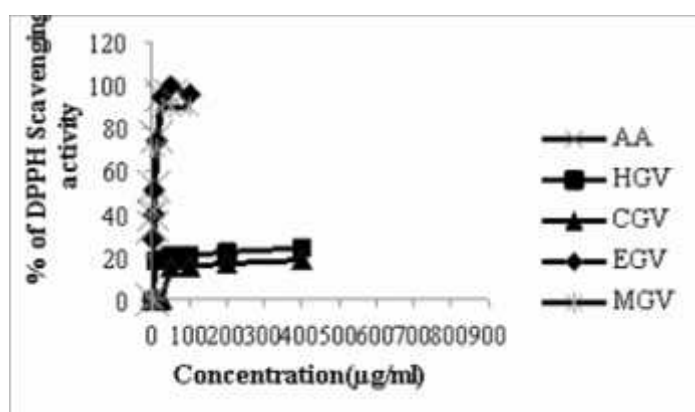


Fig. 1. DPPH free radical scavenging activity of *Glochidion velutinum*

The total phenol content in the extracts was determined using Folin-Ciocalteu reagent (FCR) based on colorimetric method at 765nm by following standard procedure (Harborne *et al.* 2009). Gallic acid was used as standard to produce calibration curve. The phenolic content was expressed as mg of gallic acid equivalents (GAE) /gm of extract. Total flavonoid content in the extracts was determined using the colorimetric method involving aluminum chloride (Chang *et al.* 2002). Quercetin was used as a reference standard and flavonoid contents of extracts were calculated as mg of QE/ gm of extract. The mean and standard errors of the phenolic and flavonoid compounds of the extracts of *G. velutinum* have been shown in Figs. 2 and 3 respectively. Methanolic and ethanolic extracts of GV had greater amount of phenolics (29.89 ± 1.22 & 17.9 ± 1.26 mg/gm, GAE) as well as flavonoids (48.12 ± 2.28 & 30.22 ± 2.84 mg/gm, QE) as compared to chloroform and n-hexane extracts. However, no remarkable differences were found in phenolic and flavonoid content of CGV and HGV.

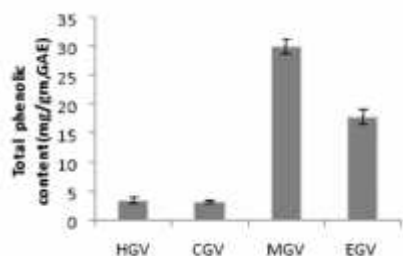


Fig. 2: Total phenolic content of extracts of *Glochidion velutinum*

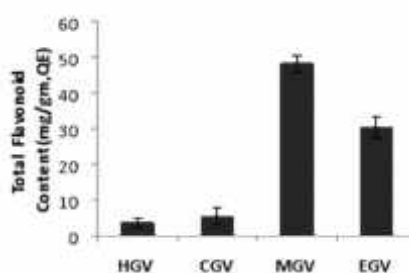


Fig. 3: Total flavonoid content of extracts of *Glochidion velutinum*

The antibacterial activity of the extracts was determined by the disk diffusion method using nutrient agar medium (Baur *et al.* 1966). Gram positive and Gram negative bacterial species were collected as pure cultures from the Department of Microbiology, Gono Bishwabidyalay. The sterile Whatman-1 filter paper disks (6mm), impregnated with 500 µg of hexane, chloroform, methanol and ethanol extract, were placed gently on the previously marked zones on the agar plates seeded in the test organisms. The plates were incubated at 37°C for 24 hour. The zones of inhibition produced by the extracts against different microorganism were measured. From the results of antibacterial activity shown in Table 2, it is evident that the methanol and ethanol extracts exhibited better activity than the hexane and chloroform extracts. Methanol extract showed mild sensitivity to *Escherichia coli*. Hexane, chloroform, and ethanol extracts did not show any sensitivity to *Escherichia coli*. No zone of inhibition has been observed in case of negative control (NC).

Table-2: Antibacterial activity of different solvent extracts of *Velutinum*

Microbes	Diameter of inhibited zone(mm)				
	HGV	CGV	MGV	EGV	NC
<i>Staphylococcus aureus</i>	7	9	18	17	nzd
<i>Bacillus subtilis</i>	8	11	21	14	nzd
<i>Pseudomonas aeruginosa</i>	8	11	17	16	nzd
<i>Shigella dysenteriae</i>	7	7	15	11	nzd
<i>Escherichia coli</i>	-	-	6	-	nzd
<i>Streptococcus pyogenes</i>	8	7	19	16	nzd

*nzd: no zone detected

Phenols are the omnipresent metabolites in plants and responsible to wide range of therapeutic uses such as antioxidant, antimutagenic, anticarcinogenic, free radical and scavenging activities. The scavenging ability of phenols is due to the presence of phenolic hydroxyl group (Ayesha Siddiqua *et al.* 2010). Antioxidants have important roles in preventing free radical damage to blood, cells, and tissues (Saleh *et al.* 2010).

The present study displayed that (Fig. 1) ethanol and methanol extracts had the highest antioxidant activity as compared to hexane and chloroform extracts. According to a study by Erkan *et al.*, (2008), there is a close relationship between antioxidant activity and total phenolic compounds of natural extracts. So the present study clearly demonstrates that all the extracts of experimental plant possess antioxidant activity. The presence of secondary metabolites has been reported to be responsible for their antibacterial properties (Adeshina *et al.* 2010). The ethanol and methanol extracts exhibited moderate antimicrobial (greater zone of inhibition) activity against microbes used in this study as compared to the hexane extract. This may be due to alkaloids and saponins being largely present in the methanol extract. The finding is consistent with the reports that nitrogen containing naturally occurring alkaloids have antimicrobial properties due to their ability to intercalate with the DNA of microorganisms (Kasolo *et al.* 2010).

From the present experiments, it is clear that antioxidant and antibacterial activities of *Glochidion velutinum* varied significantly depending on the extracting solvents. Nevertheless, further research is needed for isolation and identification of active substance present in the extracts, which could be rational for medicinal uses.

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