The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Phytochemicals and Elucidation of PolyPhenol Compounds of Aqueous-Methanol Fraction of *Desmodium velutinum* Stem Using High Performance Liquid Chromatography (HPLC)

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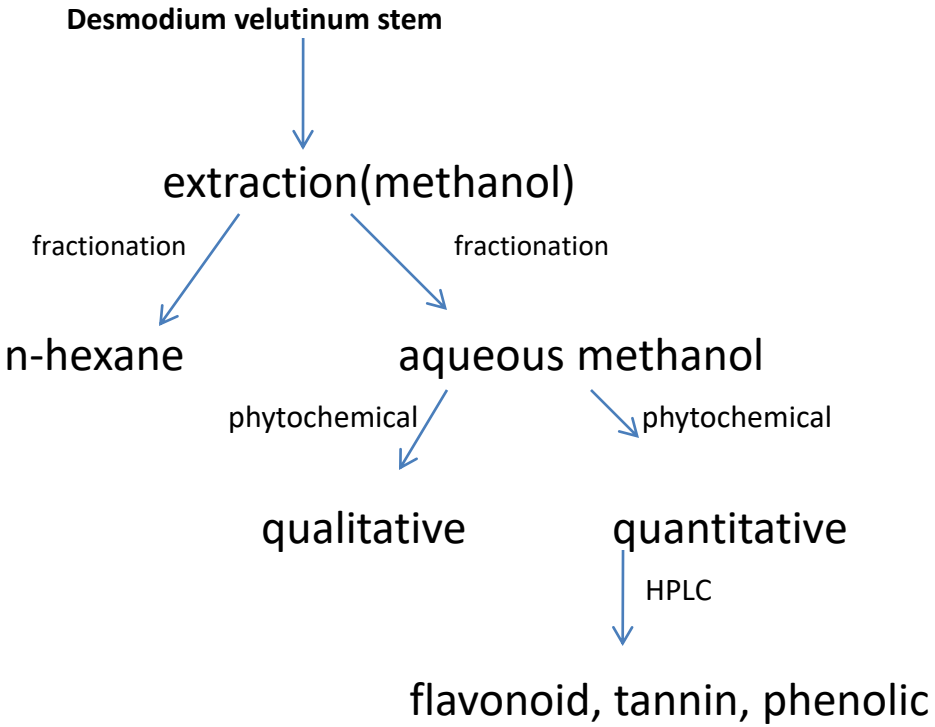
Graphical Abstract



Abstract

The aim of this study was to analyse and identify phytochemicals and bioactive constituents present in *Desmodium velutinum* stem. Fresh stems of *Desmodium velutinum* were air dried at room temperature for 3 days. The stems were pulverized into fine powder which was extracted with methanol (80% v/v). The concentrated extract was fractionated using ethylacetate, n-hexane and aqueous methanol. Phytochemical analyses and bioactive composition were done using standard methods and HPLC. The fractionation of 40 g of *Desmodium velutinum* stem methanol extract using ethylacetate gave a percentage yield of 11 %, 40 g of n-hexane yielded 8 % and 40 g of aqueous methanol gave a 16 % percentage yield. The phytochemical analyses showed the presence of tannins, flavonoids, phenolics and alkaloids with concentrations 5.02±1.22 mg/g, 2.64±0.01 mg/g, 7.02±1.18 mg/g and 3.61±0.04 mg/g respectively. The high-performance liquid chromatography (HPLC) analysis of the aqueous fraction of *Desmodium velutinum* stem showed the presence of different constituents of flavonoids, phenols, and tannins in varying concentrations. The flavonoids include gallic acid, chlorogenic acid, rutin hydrate, caffeic acid and apigenin and two (2) unidentified flavonoid compounds with different retention time. The phenols identified were ascorbic acid, p-coumaric acid, rutin, ferrulic acid, and apigenin while the tannins identified were tannic acid and gallotannin as well as eight (8) unidentified tannic compounds with different retention time. These phytochemicals identified in *Desmodium velutinum* stem possess antioxidant properties which may be used in preventing and alleviating diseases. *Desmodium velutinum* may therefore be a potential source of new drugs in orthordox medicine.

Keywords: Flavonoids; HPLC; phenol; tannin

Introduction

•The term “medicinal plants” refers to all the plants that can be used as medicines or food supplements, playing important roles in human health (Jie *et al.,* 2021). Medicinal plants are a reservoir of biologically active compounds with therapeutic properties that over time have been reported and used by diverse groups of people for treatment of various diseases (Mya *et al.,* 2019).

•Natural products, especially those derived from plants, have been used to help mankind sustain human health since the dawn of medicine. Traditional medicine has been in existence since time immemorial and has been well accepted and utilized by the people throughout history.

•Since ancient times, plants have been an exemplary source of medicines. Plant-derived medicinal products have attracted the attention of scientists around the world for many years due to their minimum side effects and positive effects on human health (Mya *et al.,* 2019).

- The usage of metabolites including dietary supplements derived from plants has increased in the couple of years. In the pharmaceutical landscape, plants with a long history of use in ethnomedicine can be a rich source of substances for the treatment of various ailments and infectious diseases.

- Medicinal plants are considered a repository of numerous types of bioactive compounds possessing varied therapeutic properties. The vast array of therapeutic effects associated with medicinal plants includes anti-inflammatory, antiviral, antitumor, antimalarial, and analgesic properties (Mya *et al.,* 2019). These therapies have become very popular in rural areas with little or no access to modern medicines and among poor people from the urban areas.

- Many plants are good source of natural medicines and bioactive compounds participating in free radical scavenging activities owing to antioxidants. Carotenoids and polyphenols for example, exhibited many beneficial effects including anti-atherosclerosis, antiaging, anticancer and anti-inflammation (Yu *et al.*, 2015).

- *Desmodium velutinum* has been reported in traditional medicine to have medicinal properties. Extracts of *D. velutinum* are used traditionally in treatment of some diseases, hence may be used as sources of pharmacological active agent in the treatment of aches and pains (Isah *et al.,* 2014). *Desmodium velutinum* (DV) serves many therapeutic purposes such as anti-diarrhoea and anti-pyretic, anti-inflammatory (Isah *et al.,* 2014), anti-nephrolithic, and anti-bacterial.

- This study was therefore aimed at analysing and identifying phytochemicals and their different constituents present in *Desmodium velutinum* stem.

Results

Percentage Yield of Methanol Extract of *Desmodium velutinum* stem
The extraction of 1000 g of *Desmodium velutinum* stem with methanol (80 %) produced an extract with a percentage yield of 3.02 %.

Percentage yield of n-Hexane fraction of *Desmodium velutinum* methanol stem extract
The fractionation of 40 g *Desmodium velutinum* stem extract using n-hexane gave a percentage yield of 8 %.

Percentage Yield of Ethylacetate fraction of *Desmodium velutinum* methanol stem extract
The fractionation of 40 g of *Desmodium velutinum* stem methanol extract using ethylacetate gave a percentage yield of 11 %.

Percentage yield of Aqueous methanol fraction of *Desmodium velutinum* stem methanol extract
The fractionation of 40 g of *Desmodium velutinum* stem methanol extract using aqueous methanol gave a percentage yield of 16 %.

Table 1:Qualitative phytochemical analysis of aqueous-methanol fraction of *Desmodium velutinum* stem

The qualitative analysis of Desmodium velutinum stem extract showed the presence of phytochemicals such as tannins, flavonoids, phenols and alkaloids. Saponins and steroids were not detected as seen in Table 1 below

Phytochemicals	Bioavailability
Tannins	++
Flavonoids	+
Phenolics	++
Alkaloids	+
Saponins	–
Steroids	–

Table 2: Quantitative phytochemical analysis of aqueous-methanol fraction of *Desmodium velutinum* stem

The quantitavive phytochemical analysis showed tannins (5.02±1.22 mg/g), flavonoids (2.64±0.01 mg/g), phenolics (7.02±1.18 mg/g) and alkaloids (3.61±0.04 mg/g) as seen in Table 2 below.

Phytochemicals	Concentration
Tannins	5.02±1.22
Flavonoids	2.64±0.01
Phenolics	7.02±1.18
Alkaloids	3.61±0.04
Saponins	–
Steroids	–

Table 3: Flavonoid compounds present in aqueous-methanol fraction of *Desmodium velutinum* stem

Peak no	Peak id	Retention time	Height	Area	Concentration (µg/100g)
1	Gallic acid	5.123	889628.125	28494840.000	112.9417
2	Chlorogenic acid	6.473	1138218.625	31056810.000	114.1053
3	Rutin hydrate	10.040	796014.313	19501428.000	18.8571
4	Caffeic acid	14.923	849314.250	119708032.000	154.3685
5	Apigenin	25.615	279715.094	21331610.000	19.6883
6	Unidentified	0.115	8514.920	37558.117	0.0171
7	Unidentified	2.198	8157.120	48607.801	0.0221

Table 4: Phenolic compounds present in aqueous-methanol fraction of *Desmodium velutinum* stem

Peak no	Peak id	Retention time	Height	Area	Concentration (µg/100g)
1	Ascorbic acid	5.332	15286.400	67977.703	66.5892
2	p-Coumaric acid	7.565	2613.647	9808.740	9.6084
3	Rutin	8.640	1929.029	12191.226	11.9422
4	Ferrulic acid	9.873	763.441	7075.135	6.9306
5	Apigenin	13.723	459.125	5032.400	4.9296

Table 5: Tannin compounds present in aqueous-methanol fraction of *Desmodium velutinum* stem

Peak no.	Peak Id	Retention time	Height	Area	Concentration (µg/100g)
1	Tannic acid	1.740	40346.414	1020381.188	65.4982
2	Gallotannin	2.815	6468.864	241135.578	15.4785
3	Unidentified	0.123	769.673	7627.600	0.4896
4	Unidentified	0.882	463.182	6874.528	0.4413
5	Unidentified	1.048	4037.654	34421.922	2.2095
6	Unidentified	1.440	13092.965	211019.313	13.5453
7	Unidentified	3.598	1260.486	4270.509	0.241
8	Unidentified	3.657	1233.851	4126.788	0.2649
9	Unidentified	3.715	1221.217	4887.666	0.2945
10	Unidentified	3.782	1190.206	3954.528	0.2538

DISCUSSION

- The phytochemical screening of *Desmodium velutinum* stem showed the presence of tannins (5.02±1.22 mg/g), flavonoids (2.64±0.01mg/g), phenolics (7.02±1.18mg/g) and alkaloids (3.61±0.04mg/g) as shown in Table 2. Alkaloids have been extensively investigated because of their biological activity and therapeutic potential. They are endowed, indeed, with several biological activities, including anti-inflammatory, antioxidant, anti-microbial, anti-cancer (Mondal *et al.,* 2019), immunomodulatory (Khan *et al*., 2020), anticholinergic, analgesic, and antiangiogenic properties (Alasvand *et al.,* 2019).
- The high-performance liquid chromatography (HPLC) analysis of the aqueous methanol fraction of *Desmodium velutinum* stem methanol extract showed the presence of flavonoids, phenols, and tannins. The flavonoids include gallic acid, chlorogenic acid, rutin hydrate, caffeic acid and apigenin and two (2) unidentified flavonoid compounds with different retention time. The phenols identified were ascorbic acid, p-coumaric acid, rutin, ferrulic acid, and apigenin while the tannins identified were tannic acid and gallotannin as well as eight (8) unidentified tannic compounds with different retention time.

- Flavonoid compounds present in *Desmodium velutinum* stem aqueous methanol fraction were caffeic acid having the highest concentration (154.3685 µg/100g), chlorogenic acid (114.1053 µg/100g), gallic acid (112.9417 µg/100g), apigenin at (19.6883 µg/100g) and Rutin hydrate having the lowest concentrations at (18.8571µg/100g). Two unidentified compounds had concentrations of 0.171(µg/100g) and 0.221(µg/100g) respectively.

- The health benefit of gallic acid is multifaceted since it expresses anti-cancer, anti-fungal, anti-viral, and antioxidant properties. (Aborehad and Osama, 2019) Its anticancer benefits are more focused because of its potential to inhibit various types of cancer cell proliferation. It displays a chemotherapeutic effect in cervical cancer cells. Sourani (2016) provided empirical evidence suggesting gallic acid inhibits lymphoblastic leukemia cell proliferation and Manerba et al. (2012) identified a byproduct of gallic acid which inhibits Triple Negative Breast Cancer cell proliferation.

- Phenolic compounds found in *Desmodium velutinum* stem aqueous methanol fraction were ascorbic acid, having the highest concentration of 66.5892 µg/100g, Rutin 11.9422 µg/100g, p-coumaric acid 9.604 µg/100g, ferrulic acid 6.9306 µg/100g and apigenin having the lowest at 4.9296 µg/100g with their different retentive time.

- According to Shalaby and Shanab (2013), ascorbic acid is an important dietary antioxidant is required for collagen synthesis by acting as a cofactor for non-heme iron αketoglutarate-dependent dioxygenases such as prolyl 4-hydroxylase. The synthesis of collagen is important primarily due to the maintenance of normal vascular function. Vitamin C stimulates the synthesis of all types of collagens by donating electrons that are needed for the hydroxylation of proline and lysine in procollagen by specific hydroxylase enzymes.

- It is also worthwhile to mention that vitamin C, under normal physiological concentrations can protect cells against oxidative damage caused by reactive oxygen species (Kasote *et al*., 2013).

- Tannin compounds present in *Desmodium velutinum* stem aqueous methanol fraction were tannic acid and gallotannin present in concentrations of 65.4982 µg/100g and 15.785 µg/100g respectively in their different retentive time.

- The presence of these phytochemicals suggest that aqueous-methanol fraction of *Desmodium velutinum* could have various medicinal values such as antidiarrhoeic, analgesic, antioxidative, hypolipidemic and antidiabetic activities (Saxena *et al.,* 2013).

Conclusion

The presence of phytochemicals present in aqueous-methanol fraction of *Desmodium velutinum* stem has shown that it could be exploited to further curb diseases and illnesses as a result of bioactive compounds which are very instrumental in traditional medicine. It should be recommended for orthordox medicine after conducting further experiments and clinical trials.