Comparative Study of Phytotoxic role of *Pimpinella Stewartii* and *Primula Denticulata*

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Abstract:

Phytochemicals are famous for their vital role in the development and protection of plants and our environment. The present research studies confirm the presence of plenty of phytochemicals including alkaloids, tannins, terpenoids, and flavonoids. The results that *P.denticulata* and *P.stewartii* possesses several classes of secondary metabolites. The preliminary phytochemical screening of *Primula denticulata* leaf extract showed that the plant is rich in various active ingredients (secondary plant metabolites). The result of the phytochemical screening revealed a strong to moderate presence of alkaloids, phenolic, flavonoids, tannins, cardiac glycosides, terpenes, Saponins, steroids and carbohydrates in *P.denticulata* .The Qualitative phytochemical screening of *Pimpenella stewartii* were studied having standered protocols for the detection of various secondary phytoconstituents to find out the medicinal potential of *P.stewartii* . Secondary metabolites like Alkaloids, Flavonoids, Tannins, Saponins, Phenol, Phenol, Diterpenoids, Triterpenoids, Glycosides, Cardiac glycosides and Coumarins were examined by using different protocols and tests such as Wagner's test, Dragendroff's test, Mayer's test, KOH (alkaline, Gelatin test. Froth test. Ferric Chloride, Copper acetate, Liebermann-Burchard reaction, Legal's test (Sodium nitroprusside), Keller Kiliani test, Ethanol + KOH were carried out. Alkaloid, Falavonide, Saponins, Phenol, Diterpenoids and Triterpenoids were highly present in the extract of *P.stewartii.* While having

Tannins, Glycosides, Cardiac glycosides and Coumarines were not present in *P.stewartii* crude extract during experiment's.

Keywords: Phytotoxic, Plants, Phytochemicals, Metabolites, Protection

Introduction:

There are various substances, produced by the plants in order to make their survival assure, compete and inhibit the growth of plants that are produced in their surrounding environment. These substances are termed as allele-chemicals. The effects of these allele-chemicals are prominent on weeds as compared to crop plants. These allele-chemicals stay within the soil for a longer duration of time and exerts inhibitory action on weeds. Herbicide resistant weed biotypes are produced when synthetic herbicides are used in an augmented quantity to control weeds **(Jabeen et al., 2013)(R. M. Ikram et al., 2020).**

A time demanding need is that some cost effective and eco-friendly strategies are designed throughout the world to cope the issue. In this scenario, a good response can be expected from phytotoxic plants as they acquire growth impeding ingredients. The researcher's response and research work in this regard is commendable. The growing interest of researchers is due to either the easier approach of distinguishing phytotoxic plant from medicinal one and secondly, the possible huge bioactive components in the medicinal plants. The use of these phytotoxic plants is observed in varied ways including direct use of their extracts as bio-herbicides, planting the plants with main crops, their use as biodegradable herbicides production etc. The mechanism of action of certain allelochemicals is entirely different from the one that are available commercially in the market. Similarly, the plant-based herbicides also possess lesser side effects. This is the reason why researchers have focused the synthesis of herbicides from plant origin.

In recent era, researchers have attempted many useful methodologies and techniques to produce crops in an augmented quantity in order to nourish the increasing population of the world. Unfortunately, growing number of weeds and bugs has decreased the production of crops everywhere in the world. Therefore, the quest for new plant-based allelochemicals that can be used in synthesizing new herbicides is the need of the day. Till now, more than 70

Japanese medicinal plants are investigated for their possible allelochemicals and still other are under exploration. 81 medicinal plants are reported from Pakistan having allelopathic potential. This research showed that aromatic plants are a major source of allele-chemicals that are present in these plants. It is to be noted that the plant's allele-chemicals are selective in their nature. Therefore, to get to know about the allele-chemical's action is a challenging job **(R. M. Ikram et al., 2020; Islam et al., 2018; Jabeen et al., 2013)**

The medicinal plant's role in treating cancer cannot be denied. The principal mean for drug synthesis is supposed to be plant origin. Due to having less adverse events and low toxicity, various plant extracts are proved to be a good cytotoxic source. Therefore almost every fourth medicine contain plant derived ingredients. Pinaceae , Asteraceae, Chenopodiaceae and Labiatae showed best results against human cervical cancer cells **(Booth et al., 2012).** Camptothecin was isolated from Camptotheca *acuminata* in mid-1950 and was exclusively used for treating cancer but later, the drug was banned in 1970's because of its severe effects on certain vital body's organs including bladder etc **(Li et al., 2017).** Presence of an anti-cancer agent, Epipodophyllotoxin, which is an isomer of podophyllotoxin, in the roots of Podophyllum species (Podophyllum peltatum L, Podophyllum emodi) was also reported **(Shah et al., 2021).** The chances that a drug contains an active plant derived compound falls between one to four drugs. The worth of the global market for botanicals and medicinal herbs is estimated about US $18 billion or more **(Booth et al., 2012)**.

Method and Material

Phytochemical Investigations

Phytochemical Investigation involved the following the steps which include:

1) **Plants Collection and Identification:**

Both experimental plants i.e. *P. denticulata* (PD) and *P. sewartii* (PS) were authenticated and submitted in Herbarium of department of Botany, University of Peshawar to Herbarium In-charge Mr. Gillani having voucher No. for PD Bot. 20075 (PUP) and for PS Bot. 20076 (PUP). The whole plant of *P. denticulate* was collected from upper Dir, Swat and Azad Jammu Kashmir

valley. While *P. stewartii* collected from Mansehra division from June to August 2017. After proper washing with tap water and then distilled water, both plants were kept in open air at room temperature under shade till all water contents were removed and both plants become dried **(Kottapalli et al., 2016).**

2) Preservation of Plants Powder:

Both plants were washed with clean water to remove dust and debris. Then cut into small pieces and shad dried. Afterwards crushing of both completely dried plants was done by electric grinder to collect powder and to use for further extraction and isolation. The powdered materials were further grinded with a porcelain pestle and mortar and kept in glass ware having close mouth and stored at 4°C in the refrigerator. Fine powder was sieved through muslin cloth, stored in polythene bottles and was kept in desiccators for analysis as per previously used method **(Mehmood Abbasi & Guo, 2015).**

Figure 1: Shows finding weight of plant powder.

3) Preparation of Crude Extract and Fractionation:

For the preparation of plant extract different solvent (methanol, butanol, ethyl acetate, DCM & *n*-hexane) were used as per standard procedure. The plant powder about 50mg was dissolved in 500ml in above mentioned solvents by coving mouth of beaker with aluminum foil to safe from contamination and the beaker was then kept on thermo shaker for 72 hours (03 days) to obtained plant extract. Subsequently the solution were filtered by using Whitman filter paper # 1. After the filtration the filtrate were passed through rotary vacuum evaporator (Hanshan, Koria) at 45°C for the separation of solvent from plant

crude material. Then the remaining material was dispensing to china dish and kept in water bath at 60°C for the complete evaporation of that solvent. As a result, the material appeared in crude form and it then kept for solidification and thus the liquid material changed into powdered when become cold. After that the powdered crude extract poured to sterile vials to protect from pathogenic attack **(Anisuzzman et al., 2017; Ghani, 1998).**

Figure 2: Shows mixing of plant powder with solvent and processing in thermo shaker

Figure 3: Shows filtration of the extract separation (Rotary vacuum evaporator) of solvent from crude extract.

Figure 4: Shows extract passing through rotary vacuum evaporator for the separation of solvents extract in water bath for complete evaporation of solvents.

Figure 5: Shows purely extract prepared

Results and Discussion:

The present research studies confirm the presence of plenty of phytochemicals including alkaloids, tannins, terpenoids, and flavonoids. The results that *P.denticulata* and *P.stewartii* possesses several classes of secondary metabolites. The phytochemical test was done by plant extract with different fractions such as DCM, ethyl acetate, butanol,n- hexane and methanolic extract. Phytochemical screening reveals that the examined plants contained various classes of a secondary group of compounds including alkaloid, Terpenoids, flavonoids, tannins, steroids, Saponins, cardiac glycosides and Anthraquinones. The highest content of phytochemicals (steroids) was found in DCM, hexane, ethyl acetate, butanol, and methanol extract.

The present outcomes were confirmed by Aslam *et al*., (2015) who also confirmed the availability of phenols, tannins, and alkaloids in several fractions of the subject plant, showed that alkaloids and tannins content increase with altitude. This unique behavior is supported by several studies conducted by researchers working on essential oil characterization of hexane fraction. Various phytochemicals present in hexane fraction are considered responsible for several therapeutic activities.

Phytochemicals are famous for their vital role in the development and protection of plants and our environment. Flavonoids are another class of natural products that reduce the risk of many chronic diseases including cancer, cardiovascular disease (CVD), neurodegenerative, microbial infections, diarrhea, antioxidants, anti-carcinogens and inflammation of tissues (Kozłowska, 2014). Likewise, tannins and alkaloids played their role as antioxidants and anticancer. Furthermore, there is ample proof of their anti-inflammatory, cicatrizant, and anti-HIV functions reported by several researchers (Bribi, 2018; Furlan, 2011).

Saponins are triterpenoid glycosides with plenty of defensive mechanisms including controlling the protozoans and mollusk's pathogens androdent types of animals. They also observed for their analgesic, anti-nociceptive, antioxidant activities, and to impair the digestion of protein, to cause hypoglycemia, and to act as antifungal and antiviral agents (Desai, 2009). Similarly, another type of steroids known as cardiac glycosides is primarily valuable in the medication of

congestive heart failure. These are potentially employedon controllingstrokein a small amount on the cardiac muscle and exert a beneficial simulation on the diseased heart (Morsy, 2017).

Anthraquinoneis a unique type of secondary metabolites that are abundantly found in nature and structurally recognizedas 9,10-dioxoanthracene. The glycosides of 9,10-dioxoanthracene also identified as anthracene 9,10-dionesare frequently found in wild plants. The Anthraquinones and related glycosides exhibited anticancer, anti-inflammatory, antimicrobial, diuretic, vaso-relaxing activities and possess wide-ranging phytoestrogen activities. Keeping these outcomes, it is suggested that Anthraquinone should be evaluated for possible clinical applicationsin the above-mentioned diseases (Chien, 2015).Terpenoidsare the largest and most widespread class of secondary metabolitesmainly found in plants. Thesenatural products had immense bioactivity including antimicrobial, antineoplastic with several pharmaceutical functions. Similarly, Steroids are organic compounds widely distributed in living species including plants, animals, and fungi. They have much biological significance in living organisms. Steroids protect the species from various diseases including inflammation, bone fracture, and cardiovascular abnormalities. Besides, these are involved in the formation of reproductive hormones in a living organism (Tholl, 2015).

Preliminary phytochemical screening of *Primula denticulata* leaf extract showed that the plant is rich in various active ingredients (secondary plant metabolites). The result of the phytochemical screening revealed a strong to moderate presence of alkaloids, phenolic, flavonoids, tannins, cardiac glycosides, terpenes, Saponins, steroids, and carbohydrates (Aslam *et al*., 2015). Likewise, Okrslar*et al*. (2007) isolated several saponins from *P. veris*with particularly considerable amounts of triterpene Saponins. These are also confirmed as expectorants.

Table 1: Detection of Phytochemicals by applying biochemical confirmatory tests

Compound Class	Test Type	*Pimpenella stewartia*	*Primula Denticulata*
Alkaloids	Wagner's test	+++	++
Alkaloids	Dragendroff's test	+++	+++
Alkaloids	Mayer's test	+++	++
Flavonide	KOH(alkaline	+++	+
Tannins	Gelatin test	---	++
Saponins	Froth test	+++	++
Phenol	Ferric Chloride	+++	+++
Diterpeniod	Copper acetate	+++	++
Triterpeniod	Liebermann-Burchard reaction	+++	++
Glycosides	Legal's test (Sodium nitroprusside)	---	---
Cardiac Glycosides	Keller Kiliani test	---	---
Coumarins	Ethanol + KOH	---	--

(-): Negative, (---): Strong Negative, (+): Weak positive, (+ +): Positive, (+ + +): strongly positive

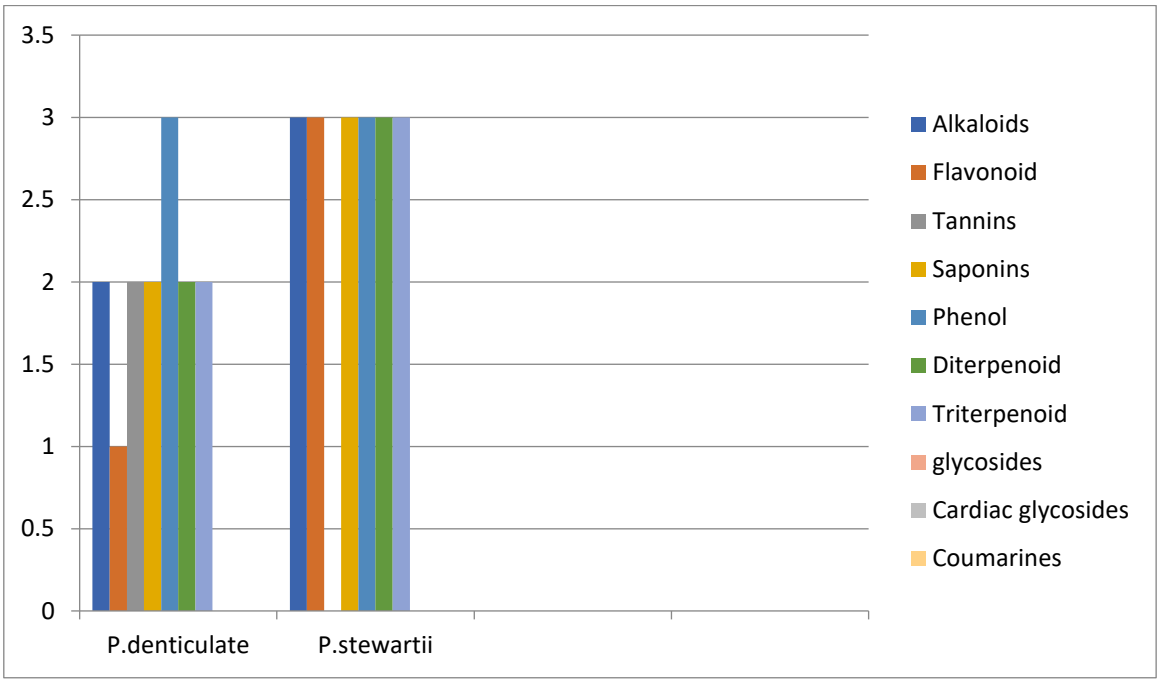

Figure 6: Detection of Phytochemicals by applying biochemical confirmatory tests

Extractive Yield:

The extractive value and color of *P.denticulata and* ***Pimpenella stewartii*** were determined and represented in Table below.

Table 2:The resultant extractive yields of selected fractions of *P. denticulata* and *Pimpenella stewartii* their phytochemical characteristics

P. denticulate				
Sr.No	**Solvents**	**Yield/total weight**	**Extract Yield (%)**	**Characteristic**
1	Methanol	72.3/180	40.12	Dark green, aromatic
2	*n*-Hexane	0.75/15.34	4.90	Dark olive green, pleasant aromatic
3	DCM	1.86/16.69	11.14	Black, burning smell
4	Et.Ac	5.13/40	13.67	Brown-black, sweat smell

5	Butanol	0.37/14.40	2.57	Dark brown, pungent
Pimpenella stewartia				
Sr.No	**Solvents**	**Yield/total weight**	**Extract Yield (%)**	**Characteristic**
1	Methanol	69.1/170	39.12	Dark green, aromatic
2	*n*-Hexane	0.76/14.31	4.50	Dark olive green, pleasant aromatic
3	DCM	1.56/15.59	10.88	Black, burning smell
4	E.Acetate	4.17/50	11.45	Brown-black, sweat smell
5	Butanol	0.37/14.40	2.17	Dark brown, pungent

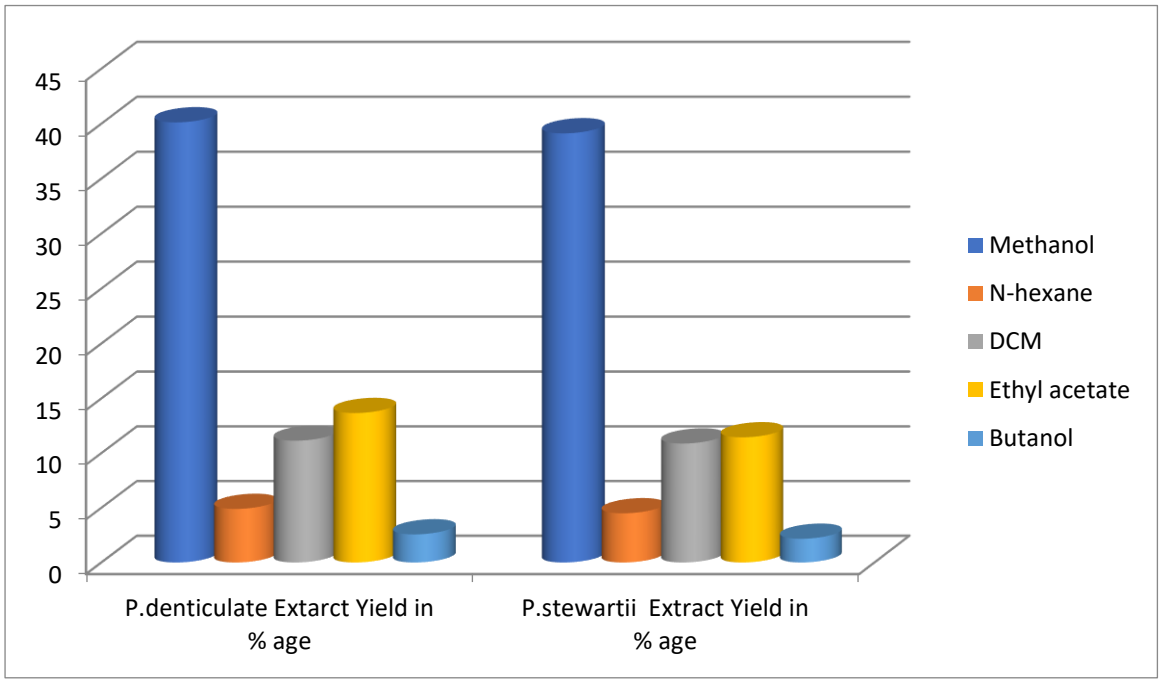

Figure 7: The resultant extractive yields of selected fractions of *P. denticulata* and *Pimpenella stewartii* their phytochemical characteristics

Phenolic Content:

The polyphenol content was determined using the adsorbing capacity of subject plant extract by using Folin- Ciocalteu reagent, using Gallic acid as standard.

The total phenolic content of the selected fraction of *P.denticulata* using different concentrations given in Table No. 4.3. The protocol consists of different concentrations (50,100,150,200) for the said analysis of the subject plant. After completing the experiment, it was observed that the highest concentration was found in ethyl acetate fraction (216.6±3.58 mg GA/g), while lowest concentration is observed in crude methanolic extract (09.23±0.71 mg GA/g). Likewise, n-hexane fraction reveals the greater concentration (17.33±1.01mg GA/g) as compared to crude methanolic extract (09.23±0.71 mg GA/g) followed by butanol (57.9±4.21mg GA/g) and DCM (66.3±2.19mg GA/g), respectively.

Similarly the *P.stewartii* fractions revealed different phenolic contents at different concentration like above mentioned. N-hexane has 17.33±1.01 total phenolic content, Ethyl acetate has 216.6±3.58 total phenolic content, Butanol has 57.9±4.21 total phenolic content, DCM has 66.3±2.19 total phenolic content and Methanolic extract has 09.23±0.71 phenolic content.

Table 3: The total phenolic content of selected fractions of *Pimpenella stewartii* and *P.denticulata*

Pimpenella stewartia		
S.No	**Fractions**	**Total phenolic content**
1	*n*-Hexane	17.33±1.01
2	DCM	66.3±2.19
3	Ethyl acetate	216.6±3.58
4	Butanol	57.9±4.21
5	Methanolic extract	09.23±0.71
P. denticulate		

S.No	Fractions	Total phenolic content
1	*n*-Hexane	18.45±2.01
2	DCM	77.2±22.25
3	Ethyl acetate	223.6±29.67
4	Butanol	55.39±17.31
5	Methanolic extract	210.53±1.81

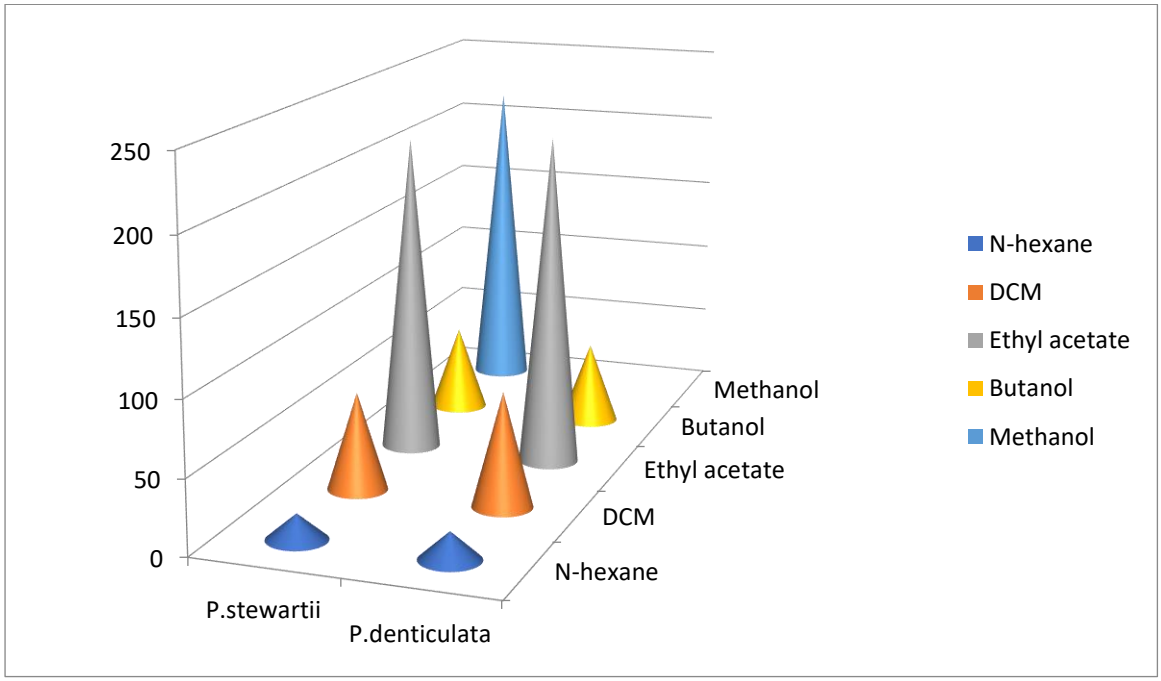

Figure 8: The total phenolic content of selected fractions of *P. denticulate and P.stewartii*

Conclusion:

Phytochemicals are famous for their vital role in the development and protection of plants and our environment. Flavonoids are another class of natural products that reduce the risk of many chronic diseases including cancer, cardiovascular disease (CVD), neurodegenerative, microbial infections, diarrhea, antioxidants, anti-carcinogens and inflammation of tissues. Likewise, tannins and alkaloids played their role as antioxidants and anticancer. Furthermore, there is ample proof of their anti-inflammatory, cicatrizant, and anti-HIV functions.

References:

- Jabeen, N., Ahmed, M., Shaukat, S. S., & Iram-Us-Slam. (2013). Allelopathic effects of weeds on wheat (Triticum aestivum L.) germination and growth. *Pakistan Journal of Botany*, *45*(3), 807–811.
- Ikram, R. M., Tanveer, A., Maqbool, R., Aziz, A., Nadeem, M. A., Nadeem, M., Abbas, T., & Raza, A. (2020). Potential allelopathic implications OFA rain-fed weed astragalus spp. on chickpea crop. *Pakistan Journal of Agricultural Sciences*, *57*(6), 1549–1555. https://doi.org/10.21162/PAKJAS/20.128
- Islam, A. K. M. M., Yeasmin, S., Qasem, J. R. S., Juraimi, A. S., & Anwar, M. P. (2018). Allelopathy of Medicinal Plants: Current Status and Future Prospects in Weed Management. *Agricultural Sciences*, *09*(12), 1569–1588. https://doi.org/10.4236/as.2018.912110
- Booth, G. M., Malmstrom, R. D., Kipp, E., & Paul, A. (2012). Cytotoxicity of selected medicinal and nonmedicinal plant extracts to microbial and cervical cancer cells. *Journal of Biomedicine and Biotechnology*, *2012*(iii). https://doi.org/10.1155/2012/106746
- Li, F., Jiang, T., Li, Q., & Ling, X. (2017). Camptothecin (CPT) and its derivatives are known to target topoisomerase I (Top1) as their mechanism of action: Did we miss something in CPT analogue molecular targets for treating human disease such as cancer? *American Journal of*

Cancer Research, *7*(12), 2350–2394.

- Shah, Z., Gohar, U. F., Jamshed, I., Mushtaq, A., & Mukhtar, H. (2021). *Podophyllotoxin : History , Recent Advances and Future Prospects*. 1–27.
- Kottapalli, S., Krishna, H., Venumadhav, K., & Jamir, K. (2016). PREPARATION OF HERBARIUM SPECIMEN FOR PLANT IDENTIFICATION AND VOUCHER NUMBER Phytoremediation View project Seed Biochemistry View project. *Roxburghia*, *6*(October), 111–119. https://www.researchgate.net/publication/309159781
- Mehmood Abbasi, A., & Guo, X. (2015). Proximate Composition, Phenolic Contents and *in vitro* Antioxidant Properties of *Pimpinella stewartii* (A Wild Medicinal Food). *Journal of Food and Nutrition Research*, *3*(5), 330–336. https://doi.org/10.12691/jfnr-3-5-7
- Anisuzzman, M., Hasan, M. M., Acharzo, A. K., Das, A. K., & Rahman, S. (2017). In Vivo and in Vitro Evaluation of Pharmacological Potentials of Secondary Bioactive Metabolites of Dalbergia candenatensis Leaves. *Evidence-Based Complementary and Alternative Medicine*, *2017*. https://doi.org/10.1155/2017/5034827
- , A. A., Abdullahi R., & M. Haque. (2020). Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacy and Bioallied Sciences, 12(1), 1–10. https://doi.org/10.4103/jpbs.JPBS_175_19
- Pandey, A., Tripathi, S., & Pandey, C. A. (2014). Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry JPP*, *115*(25), 115–119.
- Aslam, K., Nawchoo, I. A., & Ganai, B. A. (2015a). *Altitudinal variation in some phytochemical constituents and stomatal traits of Primula denticulata * Correspondence Info :* *1*(02), 93–101. https://doi.org/10.7439/ijasr
- Kozlowska, Aleksandra, and Dorota Szostak-Wegierek. "Flavonoids-food sources and health benefits." *Roczniki Państwowego Zakładu Higieny* 65.2 (2014).