Isolation and Phytochemistry of Saponin from *Tagetes erecta*

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Abstract

This research paper explores the phytochemistry of saponins, focusing on the isolation of saponins from *Tagetes erecta* (marigold). *Tagetes erecta* is a widely cultivated flowering plant with various uses in traditional medicine and agriculture. The study reviews the phytochemical composition of saponins and investigates methods for isolating and characterizing these compounds from *Tagetes erecta*. Saponins are bioactive glycosides that exhibit a wide range of pharmacological properties, such as anticancer, anti-inflammatory, and antimicrobial activities. The methods for isolating saponins include solvent extraction, column chromatography, and other techniques. This paper discusses the significance of saponins in the context of their biological properties.

Keywords: Saponins, *Tagetes erecta*, Isolation, Phytochemistry, Bioactive compounds, Traditional medicine

Introduction

Saponins are a class of secondary metabolites found in various plant species, including *Tagetes erecta* (marigold), which is widely known for its ornamental and medicinal value. Saponins are characterized by their soap-like properties, which allow them to form stable foams when mixed with water. Chemically, saponins consist of a hydrophobic aglycone (sapogenin) and a hydrophilic sugar moiety. These compounds are of great interest in the field of phytochemistry due to their diverse biological activities, which include anticancer, antimicrobial, and immunomodulatory effects (Oleszek *et al.*, 2009). This paper reviews the methods used for isolating saponins from *Tagetes erecta*.

Phytochemistry of Saponins

Saponins are primarily classified into two types based on their chemical structure: triterpenoid saponins and steroidal saponins. Triterpenoid saponins are more prevalent in the plant kingdom and are typically found in *Tagetes erecta*. These compounds are synthesized from isoprenoid precursors and consist of a triterpenoid aglycone (usually a pentacyclic triterpene) connected to one or more sugar residues.

The phytochemistry of saponins involves understanding their structural components and the variability of their molecular configurations, which are responsible for their biological activities (Zhang *et al.*, 2012). In *Tagetes erecta*, saponins have been identified primarily in the leaves, flowers, and stems. These compounds are important for the plant's defence mechanisms, acting as deterrents against herbivores and pathogens (Liu *et al.*, 2010).

Isolation of Saponins from *Tagetes erecta*

Collection and Preparation of Plant Material

Fresh flowers used for saponin extraction. The collected flowers is dried in the shade to prevent the degradation of sensitive compounds and then powdered using a mechanical grinder.

Extraction Methods

The solvent extraction methods for extracting saponins from *Tagetes erecta*, with being the most used approach. The solvent extraction technique involves the use of organic solvents such as methanol, ethanol. The dried plant powder is soaked in the solvent for a period, typically 24–48 hours, with occasional shaking to increase the extraction efficiency.

Phytochemical Tests for Saponins

Table1- Showing the phytochemical analysis of methanol extract of ***Tagetes erecta***

Chemical Tests	**Chloroform**	**Ethyl acetate**	**Ethanol**	**Water**
Alkaloids				
Mayer's reagent	-	-	-	+
Phenols/Tannins				
Ferric chloride	-	-	+	-
Gelatin Solution	-	-	+	-
Lead acetate test	-	-	+	-
Flavonoids				
FeCl3 test	-	+	+	+
Alkaline reagent test	-	+	+	+
Shinoda test	-	+	+	+

Saponins				
Foam test	+	+	+	+
Hemolytic test	+	-	+	+
Lead acetate	+	+	+	+
Spot	+	-	-	-
Saponification	+	-	-	-
Amino acids				
Ninhydrin Test	+	+	+	-
Millons Test	+	+	+	-
Xantoprotein Test	+	+	+	-
Terpenoids				
Lieberman Burchard Test	-	-	+	-
Salkowski test	-	-	+	-
Steroids				
Lieberman Test	-	+	+	-
Protein				
Biuret test	-	-	+	+

(+) Indicates 'Presence'; (-) Indicates 'Absence'

Chromatographic analysis

Column Chromatography: This technique is used for the separation and purification of the crude extract. The extract is loaded onto a chromatographic column packed with an adsorbent like silica gel or cellulose.

Solvent gradients are passed through the column to separate saponins based on their polarity (Patel *et al.*, 2014). The fractions collected from the column are then analyzed for the presence of saponins.

Characterization of Saponins

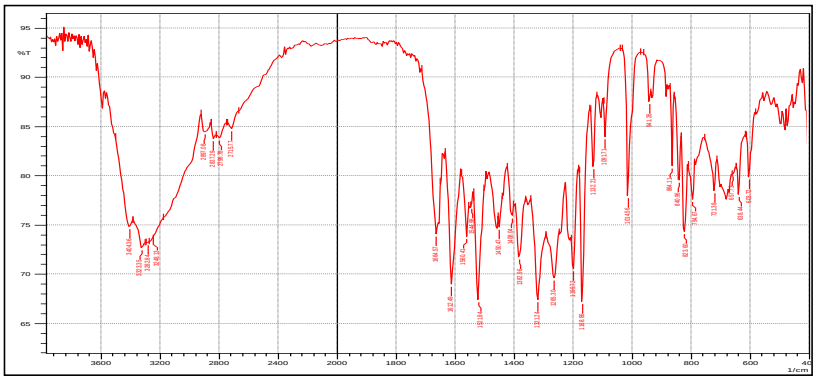
After the isolation process, the saponin fraction is subjected to various analytical techniques to confirm the presence of saponins and determine their structural features: TLC is a rapid and simple technique used to analyze saponins in crude extracts. The extract is spotted onto a TLC plate and developed using a suitable solvent system. The presence of saponins is indicated by specific colour changes after staining with iodine vapor or other reagents.Thin Layer Chromatography (TLC) is a convenient technique for analyzing and isolating saponins from *Tagetes erecta*. Saponins are amphiphilic molecules, and their separation requires solvent systems that can balance their polar and non-polar moieties. Below is a table of commonly used solvent systems for TLC of saponins:

Table-2 **Selection of mobile phase for TLC of ethanolic extract of *T.erectes*.**

MobilePhase used	**No. of Spot (UV-256)**	**No. of spot (UV-366)**	**1 % $AlCl_3$ acid reagents**	**Observation**
Ethylacetate:water(9:1)	2	2	1	No satisfactory resolution
Ethylacetate:water(7:3)	2	2	1	No satisfactory resolution
Chloroform:MeOH(9:1)	1	2	1	No satisfactory resolution
Chloroform:MeOH(7:3)	1	3	2	No satisfactory resolution
Toluene:Ethylacetate(5:5)	2	3	1	No satisfactory resolution
Toluene:Ethylacetate(6:4)	2	4	2	No satisfactory resolution
Toluene:Ethylacetate(7:3)	**3**	**5**	**2**	**Satisfactory resolution**
Toluene:Ethylacetate(7:2)	2	4	1	No satisfactory resolution

Infra red spectrum (IR) of isolated compound (Fraction 96-107) from *Tagetes erectes*

IR spectra (in KBr) showed absorption band at 3495.12 (OH, stretch), 1696.43 (C=O, stretch), 1610.55, 1537.28, 1427.20 (C=C stretch), 1309.07 (OH, *In-plane* bend), 1246-1199 (C-O stretch), 1023.81 (OH, *Out-plane* bend), 864.49 (Ar-H, *Out of plane* bend).



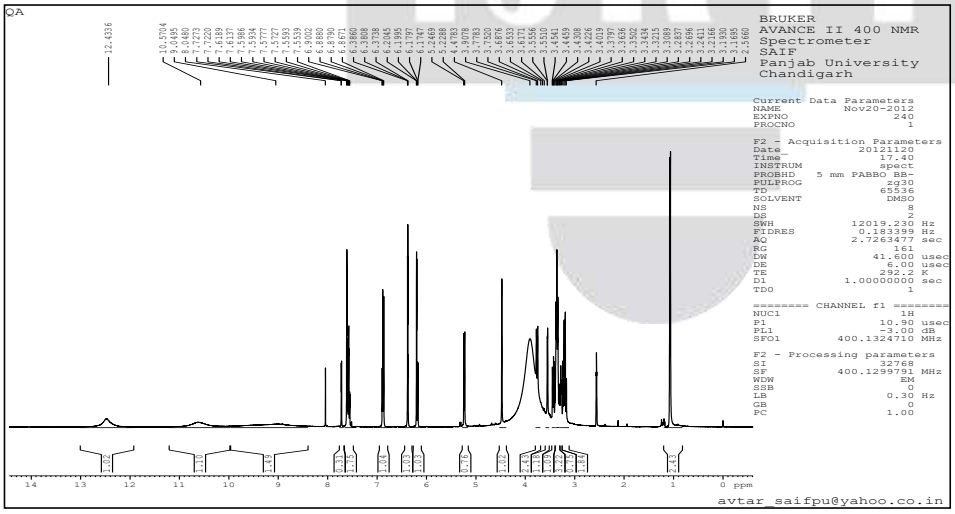
Mass Spectrometry (MS): Mass spectrometry can be used to identify the molecular weight and structure of isolated saponins. MS provides detailed information about the glycosidic linkages and aglycone moieties of the saponin molecules.The mass spectra obtained with an LC-MS system using methanol solution of the sample as shown in Graph was observed to confirm the molecular masses of the active compound. shows ESI-MS ([M-H]–) of the main Saponin.

Table 3: Mass analysis of Purified isolated compound

Peak No.	Molecular weight
01	269
02	241
03	225
04	183
05	151
06	117

Nuclear Magnetic Resonance (NMR): NMR spectroscopy is one of the most reliable techniques for determining the structural configuration of saponins. It can help identify the sugar units, aglycone structures, and linkage positions (Zhao et al., 2018). ^{1}HNMR spectra of NSBFr.1 as shown in **Graph 15**were recorded in DMSO-d_6 using a JEOL A-500 spectrometer. Chemical shifts were given in ppm and coupling constants (*J*) in Hz.

^{1}H NMR: δ 7.83 (2H, *d*, *J* = 8.8 Hz, H-2'and H-6'), 6.92 (2H, *d*, *J* = 8.8 Hz, H-3'and H-5'), 6.83 (1H, *d*, *J* = 2.1 Hz, H-6), 6.74(1H, *d*, *J* = 2.1 Hz, H-8), 6.51 (1H, *s*, H-3).



Saponins are a class of secondary plant metabolites with diverse biological properties. They occur in a great number of plant species (mainly Angiosperms), both wild plants and cultivated crops. Triterpenoid saponins are mostly found in dicotyledonous species, while many of the major steroidal saponins are synthesized by monocots (De Geyter, 2007) Secondary substances in plants are known for a long time for their medicinal and pharmacological properties. These substances are necessary for the plant to evolve in a hostile environment. The plant can indeed use its secondary metabolites to be protected against several pest, animals and pathogenic microbose (Chaieb I., 2009) .Saponins became an excellent model of study of natural substances with insecticidal effect due to their large spectrum of action and to the multitude of their physiological effect Saponins from *Tagetes erecta* possess significant bioactive properties that make

valuable for therapeutic purposes. The methods for isolating and characterizing saponins, including solvent extraction, column chromatography, and modern techniques like ultrasound-assisted extraction and supercritical fluid extraction, provide a wide range of tools for researchers. The bioactivities of saponins, such as anticancer, antimicrobial, and anti-inflammatory properties, highlight their potential for use in pharmacology and medicine. Future research should focus on optimizing extraction methods, characterizing the saponins more precisely, and exploring their therapeutic potential in clinical settings.

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