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Review Article

Pharmacognostic and Pharmacological Aspects on *Tabernaemontana divaricata* Plant

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Abstract

Tabernaemontana divaricata is a shrub commonly found in many parts of the world. Flowers of the plant have a common 'pinwheel' shape, giving it the vernacular name "pinwheel flower". This plant is highly rich in alkaloids like Conophylline, Isovoacristine, α-amyrin acetate, β-amyrin acetate etc. Gas chromatography-mass spectrophotometry also revealed that it contains chemical compounds like vitamin D, vitamin A aldehyde, vitamin E, phytol and many more. Due to the presence of chemical constituents like alkaloid and non-alkaloids, *Tabernaemontana divaricata* is used to treat various diseases. It has also been proved that the plant has antioxidant, anti-inflammatory, anti-tumour, anti-microbial and wound healing properties. Studies have found the plant extract to inhibit anticholinesterase enzyme, which makes it a potential drug in the treatment of Alzheimer's disease and Myasthenia gravis. In the current review article, we have presented the extraction, standardization, phytochemical analysis, optimization techniques, pharmacological activities of *Tabernaemontana divaricata* plant.

Keywords: *Tabernaemontana divaricate*; Extraction; Phytochemical; Standardization of Extract; Pharmacological Activities

Abbreviations

GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; GCMS: Gas Chromatography-Mass Spectrometry; LCMS: Liquid Chromatography-Mass Spectrometry; UV: Ultraviolet; TLC: Thin Layer Chromatography; AChE: Acetylcholinesterase

Introduction

Tabernaemontana divaricata is a plant under the family Apocynaceae. It was named by a German physician and botanist, J. Thomson Muller [1]. Approximately 100 species of this genus are currently found in Africa, Asia, Oceania, and the Americas [2,3]. The plant can be found in India's upper Gangetic plain, Garhwal, East Bengal, Kasia Hills, Assam, Burma, and Vishakhapatnam hills [4]. Plants which are commonly referred to as "milkweed" due to their latex content.". Other synonyms for it are *Ervatamia coronaria, Ervatamia microphyll, Ervatamia divaricata, Tabernaemontana coronaria* etc. The plant is also known as "Crape Jasmine" and "Chandani" [5,6]. It is a 1.8-2.4 m tall evergreen shrub with silvery grey bark and milky latex. The leaves are simple, opposite, elliptic or elliptic – lanceolate, smooth, glossy green, acuminate and with curly margins (Figure 1). This plant blossoms all year, having the most bloom in the spring and summer. It features waxy, pure white, ruffled-edged blossoms that are especially fragrant at night and after dark. They have 1-8 flowered cymes at the branch bifurcations, with the corolla lobe overlapping to the right in the bud. Fruit follicles are 2.5-7.5 cm long, ribbed and curved, orange or bright red within narrowed into a slender curved beak. Seeds are dull brown, minutely pitted, irregular, and enclosed in a red pulpy aril [7].

Plants in the genus *Tabernaemontana* also have high alkaloid content and are often pharmacologically active [8]. Furthermore, monoterpene indole and bis indole alkaloids are the major

classes of alkaloids within the genus. Terpenes, lactones, steroids, phenolics, and flavonoids are among the other compounds. Over 67 species have been investigated for indole alkaloids, of which 470 isolations of approximately 240 structurally different bases have been detected [2,9,10]. When used in small quantities, alkaloids are organic products of natural or synthetic origin that are basic in nature and contain one or more nitrogen atoms, usually of heterocyclic nature, and have specific physiological actions on the human or animal body. There are many indole alkaloids and their derivatives whose pharmacological activities are yet to be studied [11]. Flowers of plant contain α - amyrin acetate, β-amyrin acetate, lupeol, β–sitosterol, stigmasterol and several other alkaloids like dregamine, 20-epiervatamine, tabernamontanine, vobasine, voacangine, voacamine, flavonoid aglycones and flavanol glycosides like isovoacristine, voaphyllinehydroxyindolenine, janetine (tetrahydrolivadine), N-methylvoaphylline (hecubine), kaempferol [12,13]. Leaves of the plant contain the same alkaloids present in the flowers as well as others like α -amyrin, lupeol and their acetates, β-sitosterol, coronaridine, apparicine, ervaticine (2-acyl indole derivative), ervaticine, hyderabadine, lahoricine, mehranine, stapfinine, voacristine, voharine and a dimeric alkaloid, conophylline 17-oestradiol [14]. Roots of plant contain 2 vobasinyl-iboga bisindole alkaloids i.e., 19,20-dihydrotabernamine and 19,20 dihydroervahanine having anticholinesterase activity [15].

Figure 1: Whole Plant of Tabernaemontana divaricate.

Almost all parts of the Crape Jasmine plant can be utilised to cure a variety of ailments. Its crude extract is antimicrobial against infectious disorders like syphilis, leprosy, and gonorrhoea, as well as antiparasitic against worms, dysentery, diarrhoea, and malaria. Van Beek., *et al*. in western India claim that the latex is used for inflammation and wound healing [16]. The ash of the stem is used to treat ocular problems, the root to treat toothaches, and milky latex infused with coconut oil to treat headaches. The blossoms are soothing, aromatic, and they can be used to treat burning sensations, ophthalmitis, and dermatopathy [7]. The plant also has antioxidant, anticancer, and analgesic properties, which make it useful in the treatment of fractures, rheumatic pain, fever, and so on. It is used in many regions around the world as a tonic for the kidney, liver, and spleen, as well as to cure disorders such as pruritus, asthma, hysteria, paralysis, piles, rabies, ulceration, vomiting, and so on [17]. It has been reported that alcoholic extracts of *Tabernaemontana divaricata* root and stem have a very high inhibitory activity against AChE, implying that it may be practitioners in the field for many neurodegenerative disorders, particularly myasthenia gravis and Alzheimer's disease [16]. It has also been reported that the plant extract can treat skin diseases, aches, scabies and eye diseases and act as an antihypertensive, diuretic, emmenagogue, hair growth promoter, aphrodisiac [17].

Pharmacognostic study

Collection and authentication of plant

Fresh and healthy leaves *Tabernaemontana divaricata* were collected from Vadgaon (Charvadwadi), Pune district, India and brought to the laboratory for quantification i.e. Agharkar Research Institute, Pune, Maharashtra, India.

Extraction methods

Maceration

Preparation of extract

The dried powder of *Tabernaemontana divaricata* stems/leaves/flowers was macerated with 95% (v/v) ethanol or with respective solvent for 3 days. The supernatant was decanted, replaced with fresh media, and extracted for two more days. The extracts were pooled together and evaporated using water bath to obtain a concentrated crude extract. The yield obtained by Maceration was 37.9766% [18].

Soxhlet extraction

Preparation of extract

The fresh and disease-free leaves/stems/flowers of *Tabernaemontana divaricata* were cleaned thoroughly, shade

dried, finely powdered and subjected to extraction using Soxhlet apparatus. The plant material was defatted for 18 hours using petroleum ether (40-50°C) followed by successive extraction with distilled water, ethanol, chloroform and methanol. In order to get a crude extract devoid of solvents, the extract was concentrated by evaporating the solvent using a water bath maintained at 60-80°C at ambient conditions. The yield obtained by Soxhlet extraction was 22.556% [18].

Clevenger apparatus

Preparation of extract

Clevenger Apparatus conducts the distillation process by boiling, condensing and decantation to separate the oil. The dried powdered of *Tabernaemontana divaricata* leaves/stems/flowers were collected and put inside the round bottom flask. Water was added up to optimum level and the apparatus was fixed. Temperature was maintained between 40-50°C. Extraction process was carried out for 6-7 hours to extract the volatile oil [19].

Preliminary analysis

Determination of foreign organic matter

The foreign matter was sorted into groups by visual inspection and using a hand lens after a 100 g of plant material was spread in a thin layer. The rest of the sample was sifted through a no. 250 sieve, and dust was considered mineral admixture. Weighing the sorted foreign matter. Foreign matter of aerial parts powder of *Tabernaemontana divaricata* was found to be 0.920% w/w [20].

Determination of ash value

Determination of total ash

In an ignited and tared crucible, 2g of ground dried material was accurately weighed (usually of platinum and silica). The material was spread in an even layer and ignited by gradually raising the temperature to 500-600°c until it was white, indicating the absence of carbon. Then it was allowed to cool in desiccators before being weighed. Total ash value of *Tabernaemontana divaricata* was found to be 4.90% w/w [20].

$$\text{Total ash (\% w/w)} = \frac{(z - x)}{Y}$$

z = Weight of the crucible + ash (after complete incineration)

x = Weight of the empty crucible

Y = Weight of the material taken.

Determination of water-soluble ash

The ash was obtained as in above total ash, heated for 5 minutes with 25°c of water, and filtered. Insoluble debris was collected on ash-free filter paper, rinsed with hot water, and ignited at 450°c for 15 minutes. After that, the ash was weighed. Water soluble ash value of aerial parts of *Tabernaemontana divaricata* was found to be 2.40% w/w [20,21].

$$\text{Water soluble ash (\%w/w)} = \frac{w - a}{Y} \times 100$$

w = Weight of total ash

a = Weight of the residue

Y = Weight of the material taken.

Determination of acid-insoluble ash

The ash was obtained as in above total ash, the ash boiled for 5 minutes with 25 mL of 2M HCL acid, filtered and the insoluble matter was collected on ash-free filter paper. It was ignited, cooled in a desiccator, and weighed after being washed with hot water. Acid insoluble ash value of aerial parts of *Tabernaemontana divaricata* was found to be 0.680% w/w [20,21].

$$\text{Acid-insoluble ash (\% w/w)} = \frac{a}{y} \times 100$$

a = Weight of the residue

y = Weight of material taken.

Determination of sulphated ash-

The residue was moistened with 1mL sulphuric acid and gently heated till white fumes no longer evolved. After igniting at 800 ± 25°C, all back particles disappeared. The crucible was allowed to cool and a few drops of sulphuric acid were added and the crucible was heated. After igniting it again, the crucible was allowed to cool and was weighed. Sulphated ash value of aerial parts of *Tabernaemontana divaricata* was found to be 3.0% w/w [20,21].

$$\text{Sulphated ash (\% w/w)} = \frac{(Z - X)}{Y} \times 100$$

Z = Weight of the crucible + ash (after complete incineration)

X = Weight of the empty crucible

Y = Weight of the material taken

Determination of extractive value

The powdered material of the drug (50 g) was packed in a Soxhlet apparatus and was subjected to successive extraction with different solvents like Petroleum ether, Ethyl acetate and methanol. 25 mL of filtrate was evaporated to dryness in a tarred bottom china dish. It was then dried at 105°C and weighed.

Alcoholic extractive value of *Tabernaemontana divaricata* was found to be 12.5% w/w. Petroleum ether extractive value of *Tabernaemontana divaricata* was found to be 2.40% w/w. Ethyl acetate extractive value of *Tabernaemontana divaricata* was found to be 2.45% w/w.

Determination of loss on drying

In a Petri dish, 2 gm of powder was accurately weighed and kept in a hot-air oven at 105°C for four hours. After cooling in a desiccator, the weight loss was measured. This procedure was repeated several times until the weight remained constant. Moisture content of *Tabernaemontana divaricata* was found to be 11.0% w/w [20].

$$\text{Loss on drying (\%) (LOD)} = \frac{\text{Loss in weight}}{\text{Weight of the drug (in gms)}} \times 100$$

Phytochemical analysis - quantitative tests

Total phenolic content

The amount of total phenolics in extracts was determined according to the Folin-Ciocalteu procedure [22]. In test tubes, samples (200 uL) were placed. 1 mL of Folin-Ciocalteu reagent was added, along with 0.8 mL of sodium carbonate (7.5%). The tubes were mixed and set aside for 30 minutes. At 765 nm, absorption was measured. The total phenol found to be 36 ± 1.0 mg of GAE/ gm extract.

Total flavonoid content

The total flavonoid concentration was determined using a modified colorimetric technique [23]. The plant extract (1.0 mL) was mixed with 1 mL of distilled water and 75 uL of a 5% NaNO2 solution. 75 ul of 10% $AlCl_3.H_2O$ solution was added after 5 minutes. 5 minutes later, 0.5 mL of 1M sodium hydroxide was added. After combining the solution, it was allowed to stand for 15 minutes. At 510 nm, absorption was measured. The total flavonoid contents were found to be 10.67 ± 0.577 mg of QEE/gm extract respectively.

Total protein content

The total protein was calculated using the Lowry., *et al.* technique [24]. To each standard concentration, 0.1 mL of plant extract was added, and the volume was increased to 1 mL with distilled water. 5 mL of alkaline copper sulphate reagent was added to each test tube and left to stand at room temperature for 10 minutes. After that, 0.5 mL of Folin Ciocalteu reagent was added and incubated at room temperature for 20 minutes. At 660 nm, absorption was measured. The protein concentration was found to be 9.73 ± 0.230 mg/ml.

Phytochemical analysis-qualitative test

Qualitative tests of plant extracts of *Tabernaemontana divaricata* were performed to detect the presence of various phytochemicals including Alkaloids, Tannins, Reducing sugars, Saponins, Gums, Steroids, Triterpenoids, Flavonoids and Proteins [25,26]. Table 1 indicates overall result of phytochemical analysis *Tabernaemontana divaricata* plant.

Test for alkaloids

2 mL solution of the extract and 0.2 mL of dilute hydrochloric acid were taken in a test tube. Then 1 mL of Mayer's reagent was added. Yellow colour precipitate was not formed and that was indicated as the absence of alkaloids.

Tests for tannins

5 mL solution of the extract was taken in a test tube. Then 1 mL of 5% Ferric chloride solution was added. Greenish black precipitate was formed and indicated the presence of tannins.

Tests for reducing sugar

0.5 mL of aqueous extract of the plant material was taken in a test tube. 5 mL of Benedict's solution was added to the test tube,

boiled for 5 minute and allowed to cool spontaneously. A red colour precipitate of cuprous oxide was formed in the presence of a reducing sugar.

Test for saponins

1 mL solution of the extract was diluted with distilled water to 20 mL and shaken in a graduated cylinder for 15minute 1 cm layer of foam indicates the presence of saponin.

Test for gums

5 mL solution of the extract was taken and then Molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicates the presence of gums.

Test for steroids

1 mL solution of chloroform extract was taken and then added 1mL sulphuric acid. Red colour indicates the presence of steroid.

Test for flavonoids

Added a few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red color indicates the presence of flavonoids.

Sr. No.	Constituents	Tests	*Tabernaemontana divaricata*		
			Ethanol	Aqueous	Methanol
1	Alkaloids	Mayer's test	+	+	+
2	Tannins	Ferric-chloride test	+	+	+
3	Reducing sugars	Fehling's test	+	+	+
4	Saponins	Froth test	+	+	+
5	Gums	Molish Reagent	+	-	-
6	Steroids	Sulfuric acid test	+	+	+
7	Tri-terpenoids	Salkowski test	-	+	-
8	Flavonoids	Concentrated HCL	+	+	+
9	Proteins	Ninhydrin test	+	+	+

Table 1: Phytochemical analysis of ethanolic, aqueous and methanolic extracts of *Tabernaemontana divaricata* plant.

Standardization and optimization of extracts

Proper analytical techniques are used to ensure the quality of herbal products. The quantitative analytical technique used should be selective, sensitive, accurate, precise, reproducible, and cost-effective. HPLC (High Performance Liquid Chromatography), HPTLC (High Performance Thin Layer Chromatography), TLC (Thin-Layer Chromatography), GC-MS (Gas Chromatography-Mass Spectroscopy), LC-MS (Liquid Chromatography-Mass Spectroscopy) and CC (Column Chromatography) are the most commonly used techniques.

Gas chromatography-mass spectrometry is an analytical technique used to identify different, unknown substances present in a test sample [27]. In this method, the known compound spectrum is matched with unknown components in complex mixture.

Kalaimagal., *et al.* prepared *Tabernaemontana divaricata* flower extract for GC-MS by treating 25 g flower powder with 50 mL ethanol for 12 hours and hand-sieving it amidst sodium sulphate using Whatman Filter paper no.41. This filter paper had been dampened with 95% ethanol and sodium sulphate to operate as a filtration unit for the sediments and water in the extract. By bubbling nitrogen gas into the filtrate, it was shortened. A 2 µL extract was fed into the GC-MS system [28]. The experiment was carried out with a Perkin Elmer GC Clarus 500 instrument. The following conditions were maintained: Column-Elite-5 MS made up of 5% diphenyl or 95% dimethyl polysiloxane. Electron energy was maintained at 70 eV. Helium was used as hauler gas at 1 mL/min. Injection volume was 2 uL (10:1). Temperature of injector, inlet, and source were 250°C and 200°C, respectively. The oven was preheated to 200°C at a rate of 10°C/min to 5°C/min, with a

9 minutes hold at 280°C. Mass spectra were recorded at 70 eV, and mass scan (m/z) fragments from 45 to 450 Dalton were obtained. The GC and MS worked for a total of 36 minutes [29]. The familiar compounds present in the NIST (National Institute of Standard and Technology) band the unknown component spectrum identified by GC-MS were correlated. After comparison, the name, molecular weight, and structure of the tested components were determined [30].

After carrying out the technique, active chemical constituents were identified; they are presented in the table 2.

Sr. no.	RT	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	5.79	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene (Z)	$C_{15}H_{24}$	204	4.55
2	6.31	Cyclohexane propanoic acid, 3-oxo-, methyl ester	$C_{10}H_{16}O_3$	184	5.941
3	9.02	a-D-Glucopyranoside, O-à-D-glucopyranosyl-(1,3)-á-D-fructofuranosyl	$C_{18}H_{32}O_{16}$	504	10.80
4	10.11	Vitamin D3	$C_{27}H_{44}O$	384	0.43
5	10.84	Desulphosinigrin	$C_{10}H_{17}NO_6S$	279	8.08
6	11.6	Lactose	$C_{12}H_{22}O_{11}$	342	4.11
7	12.163	n-hexadecenoic acid	$C_{16}H_{32}O_2$	256	16.16
8	13.24	Cyclopropane tetra decanoic acid, 22-octyl-, methyl ester	$C_{26}H_{50}O_2$	394	0.85
9	14.16	9,12-Octadecadienoic acid (Z, Z)	$C_{18}H_{32}O_2$	280	15.49
10	18.51	4-(4-Methyl-2-biphenylyloxy) phthalonitrile	$C_{21}H_{14}N_2O$	310	1.40
11	21.76	9,12,15-Octadecatrienoic acid, 2,3-bis (acetyloxy) propyl ester (Z, Z, Z)	$C_{25}H_{40}O_6$	426	0.47
12	22.81	Squalene	$C_{30}H_{50}$	410	2.29
13	28.83	Cholestan-3-ol, 2-methylene-, (3á,5à)	$C_{28}H_{48}O$	400	3.55
14	30.26	Vitamin A aldehyde	$C_{20}H_{28}O$	284	2.80
15	31.14	1-Heptatriacotanol	$C_{37}H_{76}O$	536	11.36
16	31.85	Urs-12-en-24-oic acid, 3-oxo-methyl ester	$C_{31}H_{48}O_3$	468	11.72

Table 2: Active chemical constituents in *Tabernaemontana divaricata* flowers.

Muniyandi., *et al.* and Philip., *et al.* performed GC-MS analyses on *Tabernaemontana divaricata* leaves, revealing the presence of 96 phytoconstituents, 17 of which are bioactive and 11 of which have antioxidant potential. 30 g *Tabernaemontana divaricata* was treated with 300 mL of 95% ethanol in a Soxhlet apparatus for extraction. Vacuum distillation was used to filter, collect, and dry the residue. Thermal desorption (TD) system 20 was used in conjunction with GC-MS analysis (GC-MS - QP-2010 Plus, Shimadzu, Tokyo, Japan). The following were the GC-MS systems experimental conditions: Trace-5 mass spectrometry capillary standard non-polar column, 30 m length; internal diameter: 0.25 mm; film thickness: 0.25 m. The flow rate of the mobile phase (carrier gas: helium) was fixed to 1.2 mL/min. During the gas chromatography phase, the temperature programme was set to 80°C, which was elevated to

250°C at a rate of 10°C /min, and the injection volume was 1 uL. The Wiley Spectral Library Search Software was used to compare the findings of samples dissolved in chloroform that were run entirely at a mass-to-charge ratio (m/z) range of 50-650 [31].

The detected phytoconstituents with their biological activities were elaborate in table 3.

Liquid-Chromatography-Mass Spectroscopy is an analytical technique used for detection of amino acids was carried out from various extracts of *Tabernaemontana divaricata.*

Rajashree., *et al.* employed Liquid Chromatography-Mass Spectroscopy (LC-MS) to detect amino acids in *Tabernaemontana divaricata*. Amino acids are the building blocks of proteins, and determining their sequence in a protein is critical for the development of novel therapeutics. The extracts were prepared by crushing 0.5g of powdered material in a mortar and pestle with different solvents (2 mL each), such as acetone, ethanol, and water. They then filtered the extract and used it for LC-MS injection. Shimadzu LC-MS with UV detector was used. C_{18} column, 50 x 4.6 mm column was employed.

The mobile phase consisted of –

- 10 mM Ammonium Formate in water + 0.1% Formic acid
- Acetonitrile + 5% Solvent A + 0.1% Formic acid.

Injected Volume – 5 µL and Flow Rate - 1.20 mL/min. Gradient programme used - 5% B to 100% B in 3.5 minute, Hold till 0.50 min, At 4. 10minute B concentration is 5% up to 5 minutes. The spectra obtained for each amino acid detected with varying retention times were then compared with standard amino acid spectra and the amino acids were evaluated. They were confirmed with their retention times and $\{M+1\}^+$ peaks [32].

The table 4 given below showed the LC-MS data for standard amino acids in leaves extract;

Phytoconstituent	Bioactivity
2-Pyrrolidinone	Antioxidant and Anticancerous
4H-Pyran-4-one,2,3-dihydro-6-methyl	Antiproliferative, Antimicrobial and Anti-inflammatory
Retinol, acetate	Antioxidant
Tetra decanoic acid	Antioxidant and Nematocidal
3,7,11,15-Tetramethyl-2-hexadecan-1-ol	Antimicrobial, Anti-inflammatory, Antidiuretic and Anticancerous
Hexadecanoic acid, methyl ester	Antioxidant, Hypocholesterolaemia
n-Hexadecanoic acid	Antioxidant, Hypocholesterolaemia, Antiandrogenic and Haemolytic
Hexadecanoic acid, ethyl ester	Antioxidant, Hypocholesterolemic
Phytol	Antioxidant, Antimicrobial, Anticancerous and Anti inflammatory
Octadecanoic acid	Antimicrobial
9,12,15-Octadecatrienoic acid, ethyl ester, (Z, Z, Z)	Antioxidant, Antimicrobial Anticancerous and Hypocholesterolaemia
Vitamin E	Antioxidant, Anti-inflammatory, Antitumorigenic, Antileukemic, Anticoronary, Antidiabetic, Antiulcerogenic and Antidermatitic
Ergost-5-en-3-ol, (3Beta,24R)	Antimicrobial and Anti-inflammatory
Stigmasterol	Antimicrobial, Anti-inflammatory, Anticancerous, Anti-arthritic and anti-asthmatic
Stigmast-5-en-3-ol (B-Beta)	Antioxidant, Antimicrobial and Anti-inflammatory

Table 3: Biological activities reported for the phytoconstituents that were detected in the present study by screening of the extract of *Tabernaemontana divaricata* leaves.

Name of amino acid	RT for standard amino acids	RT for plant extract			$[M+1]^+$	$[M+Na]^+$
		B^1	B^2	B^3		-----
2-Amino butyric acid	0.630	0.630	-----	0.660	104	-----
Proline	0.62	-----	0.65	0.657	116	-----
Arginine hydrochloride	0.602	-----	0.612	-----	174	-----
Iso-Leucine	1.183	-----	-----	1.2	132	-----

Cysteine monohydrochloride	0.599	-----	-----	0.55		145
Leucine	0.975	-----	-----	1.02	132	
B^1 = Alcohol extract, B^2 = Acetone extract, B^3 = Water extract, RT = Retention time in minutes						

Table 4: LC-MS data for standard amino acids and leaves extracts.

Thin layer chromatography and column chromatography

Compounds were isolated from the acid insoluble/acid soluble extract by using column chromatography and fractions were monitored on TLC. To obtain free flowing material, dried basified extract was diluted in a little amount of methanol and then adsorbed on a weighed amount of silica gel-G (60–120) slurry. A clean and dry column was chosen. Diameter of column is 6 cm and Length of column is 112 cm. A cotton plug was placed at the bottom of the column. The column was filled with a slurry of silica gel-G obtained by suspending it in the solvent (chloroform). Afterwards, the adsorbed extract was charged into the column. Firstly, column was eluted with petroleum ether. Then column was eluted with the solvent by gradually increasing the percentage of ethyl acetate in petroleum ether. Each 250 mL fraction was collected and concentrated using rotavapor. A total of 239 fractions were obtained, and TLC was performed on all 239 fractions using various developing solvents. The fractions were grouped based on the TLC pattern they displayed. Developing solvents used for the TLC in various proportions -chloroform: methanol or Pet ether: acetone: diethyl amine. Spraying the chromatogram with Dragendroff's reagent Fractions 70-81 eluted in 10% ethyl acetate revealed the spots. It was then dried with a rota-evaporator and subjected to subsequent processing. The compound's melting point was determined to be 131.6°c. Based on the TLC pattern, melting point, and spectroscopic data, the molecule was identified as β-**s**itosterol [33].

Pharmacological activities

Anti-acetylcholinesterase effect

Acetylcholinesterase inhibitors (AChE-Is) derived from *Tabernaemontana divaricata* are the standard treatment for Alzheimer's disease. Ingkaninan., *et al.* (2006) used the Ellman's colorimetric technique to report on the bioassay-directed fractionation of *Tabernaemontana divaricata* various parts (blossoms, leaves, stems, and roots) on AChE action. The stem and root extracts inhibited the most, while the leaves and bloom extracts inhibited the least. These observations prompted the removal of four AChE inhibitors from this plant's root concentrate, including two new bisindole alkaloids, 19,20-dihydrotabernamine and 19,20-dihydro-ervahanine A, as well as two known bisindole alkaloids, conodurine and tabernaelegantine A [34]. This group also reported *in vivo* effects of ethanolic extracts of *Tabernaemontana divaricata* roots on AChE inhibitory effects and Fos (marker of neuronal activity) expression on neuronal activity in the cerebral cortex. The extracts increased Fos expression while inhibiting AChE in the cerebral cortex, resulting in increased neuronal activity [35]. In (2010): this group published a study on the effects of *Tabernaemontana divaricata* root extract b-amyloid 25-35 peptide on cognitive deficits in mice. Root extract was found to improve memory impairment and reduce AChE activity in the brain induced by A_{25-35} peptides in mice [36]. An alkaloidal extract from *Tabernaemontana divaricata* stem, increased acetylcholine levels in Alzheimer's patients, according to Chaiyana., *et al.* They isolated 3'-R/S-hydroxyvoacamine based on these findings, which was discovered to be a non-competitive inhibitor of AChE with an IC50 value of 7.001.99 M [37,38].

Cytotoxicity effect

According to Thind., *et al. Tabernaemontana divaricata* was found to have cytotoxic activity against HCT-15 (colon), HT-29 (colon), 502713 (colon), MCF-7 (breast), and PC-3 (prostrate) cell lines. He found that the leaf extracts with cytotoxicity mechanisms, which included hydroxyl radical scavenging and topoisomerase inhibitory activities (hexane, chloroform, ethyl acetate and methanol). Only one colon cancer cell line (502713) was found to be effective with the ethyl acetate extract, whereas all three colon cancer cell lines were found to be effective with the chloroform extract. Furthermore, the ethyl acetate extracts inhibited topo-II selectively in the topoisomerase II relaxation assay [39]. Conophylline, a bis-indole alkaloid isolated from *Tabernaemontana divaricata,* was found to induce b-cell differentiation in rat pancreatic acinar carcinoma cells and in cultured rat pancreatic tissue by Zhang., *et al*. in 2013 [40]. In the same year, Bao., *et al*. isolated five new cytotoxic vobasinyl-ibogan-type bisindole alkaloids from

Tabernaricata aerial parts, tabernaricatines A-E, tabernaricatines F-G, and 24 known indole alkaloids [41]. Rumzhum., *et al.* used a brine shrimp bioassay to screen *Tabernaemontana divaricata* leaf extracts [42]. The study's findings revealed potential cytotoxicity when compared to the positive control, vincristine sulphate [43].

Antifertility activity

Tabernaemontana divaricata leaves and flowers extracts were found to have an antifertility effect in male rats and female albino mice by Jain., *et al.* and Mukhram., *et al.* [44,45]. Male rat reproduction was inhibited by the extracts in a dose-dependent manner. The effect may inhibit gonadotropin release, which may be responsible for the decrease in testosterone production, resulting in spermatogenesis changes. The extract treatment significantly reduced oestradiol secretion in female albino mice during the oestrous stage of the cycle, which could be due to the decline in the release of luteinizing hormone and follicle stimulating hormone, resulting in hormonal imbalance. These research results could imply that *Tabernaemontana divaricata* leaf and flower extracts have an anti-fertility effect.

Anti-inflammatory activity

In male albino mice, Jain., *et al*. found that *Tabernaemontana divaricata* leaf extract had anti-inflammatory activity. The hexane fraction, which contains a lot of flavonoid compounds, had a lot of anti-inflammatory activity. This fraction was also more active than the positive drug indomethacin [46].

Antifungal activity

Singh., *et al*. investigated the antifungal activity of a biologically active compound derived from *Tabernaemontana divaricata*. Coronaridine was isolated and identified as a major Tabernaemontana genus compound from an ethanolic extract of *Tabernaemontana divaricata*. Coronaridine had significant antifungal activity against *Penicillium chrysogenum* while compared to nystatin [47].

Anti-gastrointestinal activity

Khan., *et al*. looked into the effects of a methanol extract from *Tabernaemontana divaricata* flowers on the gastrointestinal system. A rat pyloric ligation-induced gastric ulceration model was used in the study, as well as the standard drug omeprazole. The extract reduced the amount of gastric secretion, free and total acidities, ulcer index, and pH of gastric secretions produced. The standard drug, omeprazole, provided 89.8% protection, while the extract provided 79.5% [48]. Khan., *et al.* looked at the methanol extract from *Tabernaemontana divaricata* flowers at different concentrations (125, 250, and 500 mg/kg). Gastric ulcers were tested using inducers such as aspirin and ethanol [49]. Misoprostol was used as a standard positive control. Several parameters were measured and showed a reduced index when treated with extracts, including catalase, superoxide dismutase, mucin, and total protein. It's been suggested that the extracts gastrointestinal results were attributed to an antioxidant pathway.

Anti-diabetic activity

Tabernaemontana divaricata methanol extract was tested for anti-diabetic activity in diabetic rats induced by alloxan. The findings revealed significant anti-diabetic activity, as well as a decrease in the effect of oxidative damage in rats. [61] The extracts showed a consistent mechanism to the positive control drug, glibenclamide. Methanol extract, according to Kanthlal., *et al.* may stimulate or produce stem cells in test animals' pancreas by activating insulin receptors [50]. Conophylline, a compound commonly isolated from several *Tabernaemontana* species, has been shown to have anti-diabetic properties. Conophylline was also effective at inducing activity in AR42J cells, which triggered endocrine cell modification [51]. Diabetic rats were given the same compound to test. Increased plasma levels were observed in normal and streptozotocin-induced diabetic rats, as well as a significant reduction in blood glucose levels, stating antidiabetic activity. The compound conophylline was found to rapidly induce β-cells differentiation in rat pancreatic acinar carcinoma cells and cultured rat pancreatic tissues resulting in differentiation into insulin-producing cells.

Anti-cancer activity

Several AgNps studies and research been carried out on *Tabernaemontana* species, with *Tabernaemontana divaricata* being the most studied. Different *Tabernaemontana divaricata* extracts were used for biosynthesis, characterization, and cytotoxicity screening against MCF-7 cell lines in the study by Devaraj., *et al*. The average particle size ranged between 22.85 and 22.85 nm, and the biosynthesized nanoparticles were found to be possibly cytotoxic to human breast cancer cells (MCF-7) [52]. These nanoparticles are highly likely to be used on a regular basis in a variety of fields, such as medicine and cosmetics. In another study, Anbukkarasi.,

et al. used *Tabernaemontana divaricata* extracts to biosynthesize nanoparticles, and the resulting biosynthesized nanoparticles were tested *in vivo* to prevent the formation of cataracts in Wistar rat pups during selenite-induced cataractogenesis. Rats treated with AgNps had fewer lenticular alterations than plant extract-treated rats. According to the findings, biosynthesized AgNps derived from *Tabernaemontana divaricata* extracts may limit selenite-induced cataractogenesis *in vivo* while maintaining lenticular calcium homeostasis by avoiding changes in key lenticular proteins [53].

All biological acti6vities of *Tabernaemontana divaricata* summarized in table 5.

Biological activities	Plant Part	Extract/Compound	Cell line/methods/models
Anti-acetylcholinester-ase effect	Leaf, flower, root, stem, latex	Methanol, Ethanol, Phosphate Buffer, Petroleum Ether, Dihydrotabernamine, 19,20-Dihydro-Ervahanine, Conodurine, Tabernaelegantine, Voafinine, Voalenine, N-Methylvoafinine, Voafinidine, Conophylline, Conophylline	Ellman's method
Antioxidant effect	Leaf, Flower, Stem, Root and Latex	Methanol, Aqueous, Ethanol, Petroleum Ether, Hexane, Chloroform and Ethyl Acetate, Ethyl-4-N-Octyl Benzoate, Ethyl-4-N-Decyl Benzoate.	FRAP, DPPH method-*in vitro*, Hydrogen peroxide (H_2O_2) free radicals, superoxide anion Radical scavenging, NO, ABTS, H_2O_2 scavenging, Aβ25-35peptide, Novel object recognition test (NOR), crystal violet staining and lipid peroxidation.
Anti-inflammatory effect	Flower, Leaf, Stem and Aerial Parts	Ethanol, Aqueous, Ethyl Acetate, Methanol and Hexane Fraction.	Carrageenan and Formalin Induced—mice models, Reduction of interleukin (IL)-6 Secretion and tumour necrosis factor (TNF)-α Production, Wistar rat Models and reduced croton Oil-induced edema in mice models.
Anti-fungal effect	Flower and Leaf	Ethanol, Methanol and Aqueous.	*P. chrysogenum*, *Malassezia furfur* and *in vitro* Poisoned food technique.
Anti-bacterial effect	Leaf, Bark, Petal, Flower, Twig, and root	Ethanol, Chloroform, Petroleum Ether, Diethyl Ether, Methanol, Aqueous, Acetone, Alkaloids, 5-Oxocoronaridine, Dichloromethane, Ethyl Acetate.	*K. pneumoniae, S. aureus*, *S. saprophyticus, S. agalatiae, Streptococcus pyogenes*, *E. faecalis, S. typhi, E. coli, Shigella boydii, Shigella dysenteries, P. aeruginosa, B. cereus, Klebsiella sp., Streptococcus uberis*, *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 35657), *Salmonella typhimurium* (MTCC 441), *S. flexneri* (ATCC 29508), *S. aureus* (ATCC25923) *Enterobacter* spp., *Salmonella* sp., Corynebacterium diphtheriae (AP596), *S. aureus* (MTCC 96), *B. subtilis* (MTCC 441), *B. pumilis* (8241) *P. aeruginosa* (AP585 NLF), *K. pneumoniae* strains, *Salmonella Paratyphi, Lactobacillus*, *P. vulgaris, Enterobacter aerogenes*

Cytotoxicity, *In-vitro* antioxidant, anticataractogenic, *In vivo* anticataractogenic	Leaf	Aqueous and Ethanol	Human Breast Cancer Cell Line-MCF-7
Anti-cancer effect	Leaf, Root, stem, Flower, Aerial parts and Whole plant	Chloroform, Petroleum Ether, Methanol, Ethyl Acetate, Hexane, Ethanol, Hydro Alcohol, Acetone, Aqueous, Vobasine, Dichloromethane, Chloroform-5-Oxocoronaridine, 3-Oxocoronaridine, Coronaridine, Ibogamine, Hecubine, Tabernamontanine, Voacamine, Tabernaricatines A-F, Bisindole Alkaloids, 16-Decarbo-Methoxyvoacamine, Tabernaecorymbosine A, Isovoacangine, Heyneanine, Voacangine, 19-Acetonyliso-Voacangine, Vincadiffine, Difforlemeine, Vophylline, Voacangine Hydroxy Indolenine, Voacristine, 19S-Heyneanine, 19S-Voacangarine	Human colon carcinoma (HCT-15, HT-29,502713), human breast adenocarcinoma (MCF-7), human prostate cancer (PC-3), leukaemia (HP-1), brine shrimp lethality, lung carcinoma large cell (COR-L23), laryngeal carcinoma (Hep-2), Vero cells, sarcoma 180, cloned Chinese hamster lung fibroblast (V79 cells), human colon cancer (HT-29), human small cell lung carcinoma (A-549), human hepatic cancer (HepG-2), human and rat normal skeletal muscle cell cultures (L6), renal cell carcinoma in Wistar rats, human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), colon cancer (SW480), human breast cancer (BC1), human oral epidermoid carcinoma (KB), KB drug-resistant strain (KB-V1), human prostate cancer (LNCaP), human lung cancer (Lu1), murine, lymphocytic leukemia (P388) human glioma (U373), human epidermoid carcinoma (A431), human colon cancer (Col2), human fibrosarcoma (HT), human melanoma (Mel2), hormone dependent breast cancer (ZR-75-1), Kl3-V1 cells.

Table 5: Biological activities of *Tabernaemontana divaricate.*

Conclusion

The presence of several phytochemical components and pharmacological properties of *Tabernaemontana divaricata* are discussed in this review. The pharmacological activities reported in this review confirm that this plant have a high therapeutic value, with a leading capacity for the future development of a new, safe and effective herbal remedy. The pharmacological activities of this plant may be due to alkaloids and non-alkaloids isolated from it. Reported research has found that bioactive monoterpene indole alkaloids derived from nature play a vital role in human health, nutrition, and disease prevention. Furthermore, bioactive components have demonstrated a wide range of biological effects, including antimicrobial, antioxidant, anti-inflammatory, anticholinesterase, anticancer, anti-diabetic, anti-hypertensive, anti-fungal, wound healing, and analgesic effects.

Bibliography

1. Van Beek T., *et al*. "*Tabernaemontana* L. (Apocynaceae): A review of its taxonomy, phytochemistry, ethnobotany and pharmacology". *Journal of Ethnopharmacology* 10 (1984): 1-156.

2. Silveira D., *et al*. "*Tabernaemontana* Species: Promising Sources of New Useful Drugs". *Studies in Natural Product Chemistry* 54 (2017): 227-289.

3. Clarissa MN., *et al*. "Major Bioactive Alkaloids and Biological Activities of *Tabernaemontana* Species (Apocynaceae)". *Plants* 10 (2021): 313.

4. Kirthikar KR and Basu BD. *Indian Medicinal Plants* (1998): 577-578.

5. Rapini A. "Taxonomy "under construction": Advances in the systematics of Apocynaceae, with emphasis on the Brazilian Asclepiadoideae". *Rodriguesia* 63 (2012): 075-088.

6. Athipornchai A. "A Review on *Tabernaemontana* spp.: Multipotential Medicinal Plant". *Asian Journal of Pharmaceutical and Clinical Research* 11 (2018): 45-53.

7. Warrier PK., *et al*. Indian Medicinal Plants. Madras: Orient Longman Ltd (1996): 232.

8. Silveira D., *et al*. "*Tabernaemontana* Species: Promising Sources of New Useful Drugs". Studies in Natural Product Chemistry Elsevier: Amsterdam, The Netherlands 54 (2017): 227-289.

9. Marinho FF., *et al*. "Brazilian *Tabernaemontana* genus: Indole alkaloids and phytochemical activities". *Fitoterapia* 114 (2016): 127-137.

10. Zhu JP., *et al*. "Chemotaxonomic study of the genus *Tabernaemontana* (Apocynaceae) based on their indole alkaloid content". *Plant Systems and Evolution* 172 (1990): 13-34.

11. Pratchayasakul W., *et al*. "Ethnobotany and ethnopharmacology of *Tabernaemontana divaricata*". *Indian Journal of Medical Research* 127 (2008): 317-335.

12. Rothe SP., *et al*. "Medicinal significance of ornamental plants in human welfare". *Journal of Experimental Sciences* 2.3 (2001): 30-32.

13. Henriques AT., *et al*. "Ervatamia coronaria: Chemical constituents and some pharmacological activities". *Journal of Ethnopharmacology* 50 (1996): 19-25.

14. Jain S., *et al*. "Antifertility effect of chronically administered *Tabernaemontana divaricata* leaf extract on male rats". *Asian Pacific Journal of Tropical Medicines* 5 (2012): 547-551.

15. Ingkaninan K., *et al*. *Journal of Pharmacy and Pharmacology* 58.6 (2006): 847-852.

16. Van Beek TA., *et al*. "*Tabernaemontana* L. (Apocynaceae): A review of its taxonomy, phytochemistry, ethnobotany and pharmacology". *Journal of Ethnopharmacology* 10.1 (1984) 1-156.

17. Warrier PK., *et al*. Indian Medicinal Plants Madras: Orient Longman Ltd. (1996): 232.

18. Abah SE and Egwari LO. "Methods of extraction and antimicrobial susceptibility testing of plant extracts". *AJBAS* 3.5 (2011) 205-209.

19. Evans, W.C. "Trease and Evans, Pharmacognosy". Toronto: Harcourt Pub Ltd., 16th edition, (2002).

20. Rathaur B., *et al*. "Phytochemical analysis of *Tabernaemontana divaricata*". *Journal of Pharmacognosy and Phytochemistry* 9.2 (2020) 1283-1291.

21. Kokate CK., *et al*. "Pharmacognosy". Nirali Prakashan, 39th Edition, (2007).

22. Singleton VL and Rossi JA. "Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents". *American Journal of Enology and Viticulture* 16 (1965): 144-158.

23. Bao J., *et al*. "Anthocyanins, flavonol and free radical scavenging activity of Chinese bayberry (Myrica rubra) extracts and their color properties and stability". *Journal of Agricultural and Food Chemistry* 53 (2005): 2327-2332.

24. Lowry OH., *et al*. "Protein measurement with the Folin phenol reagent". *Biological Chemistry* 193 (1951): 265-275.

25. Thombre R., *et al*. "Evaluation of phytoconstituents, antibacterial, antioxidant and cytotoxic activity of vitex negundo And *Tabernaemontana divaricata*". *International Journal of Pharma and Bio Sciences* 4.1 (2013): 389-396.

26. Sharker SM., *et al*. "Phytochemical and antinociceptive study of leaves of *Tabernaemontana divaricata*". *Journal of Medicinal Plants* 5.2 (2011): 245-247.

27. Sparkman DO., *et al*. "Gas Chromatography and Mass Spectrometry: A Practical Guide". 2nd edition, (2011).

28. Paranthaman R., *et al*. "GC-MS Analysis of phytochemicals and simultaneous determination of flavonoids in *Amaranthus caudatus* (Sirukeerai) by RP-HPLC". *Journal of Analytical and Bioanalytical Techniques* 3.5 (2012): 1-4.

29. Kumaravel S., *et al*. "GC-MS Study on Microbial degradation of Lindane". *International Journal of Applied Chemistry* 63 (2010): 363-366.

30. Arirudran B., *et al*. "GC/MS determination of bioactive components from *Cuminum cyminum* L". 7 (2018): 1671-1683.

31. Anbukkarasi M., *et al*. "Gas Chromatography - Mass Spectrometry Analysis and In vitro Antioxidant Activity of the Ethanolic Extract of the Leaves of *Tabernaemontana divaricate"*. *Pharmacognosy Journal* 8.5 (2016): 451-458.

32. Fowden L. "Plant amino acid research in retrospect: From Chibnall to Singh". *Amino Acids* 20 (2001): 217-224.

33. Harborne JB. "Phytochemical Methods- A Guide to Modern Techniques of Plants Analysis". Chapman and Hall, U.K, 5th edition, (1998).

34. Ingkaninan K., *et al*. "Vobasinyl-iboga bisindole alkaloids, potent acetylcholinesterase inhibitors from *Tabernaemontana divaricata* root". *Journal of Pharmacy and Pharmacology* 58.6 (2006): 847-852.

35. Chattipakorn S., *et al*. "*Tabernaemontana divaricata* extract inhibits neuronal acetylcholinesterase activity in rats". *Journal of Ethnopharmacology* 110.1 (2007): 61-68.

36. Nakdook W., *et al*. "The effects of *Tabernaemontana divaricata* root extract on amyloid β-peptide25-35 peptides induced cognitive deficits in mice". *Journal of Ethnopharmacology* 130.1 (2010): 122-126.

37. Chaiyana W., *et al*. "Characterization and in vitro permeation study of microemulsions and liquid crystalline systems containing the anticholinesterase alkaloidal extract from *Tabernaemontana* divaricate". *International Journal of Pharmaceutics* 452.1-2 (2013): 201-210.

38. Chaiyana W., *et al*. "3′-R/Shydroxyvoacamine, a potent acetylcholinesterase inhibitor from *Tabernaemontana* divaricate". *Phytomedicine* 20 (2013): 543-548.

39. Thind TS., *et al*. "Studies on cytotoxic, hydroxyl radical scavenging and topoisomerase inhibitory activities of extracts of *Tabernaemontana divaricata* (L.) R.Br. ex roem. and schult". *Food Chemistry and Toxicology* 46.8 (2008): 2922-2927.

40. Zhang HR., *et al*. "Conophylline promotes the proliferation of immortalized mesenchymal stem cells derived from fetal porcine pancreas". *Journal of Integrative Agriculture* 12.4 (2013) 678-686.

41. Bao MF., *et al*. "Cytotoxic indole alkaloids from *Tabernaemontana* divaricate". *Journal of Natural Products* 76 (2013): 1406-1412.

42. Rumzhum NN., *et al*. "Antioxidant and cytotoxic potential of methanol extract of *Tabernaemontana divaricata* leaves". *International Current Pharmaceutical Journal* 1.2 (2012): 27-31.

43. Zulkefli HN., *et al*. "Antioxidant activity of methanol extract of Tinospora cripa and *Tabernaemontana* corymbosa". *Sains Malaysia* 12.4 (2013): 697-706.

44. Jain S., *et al*. "Antifertility effect of chronically administered *Tabernaemontana divaricata* leaf extract on male rats". *Asian Pacific Journal of Tropical Medicine* 11.5 (2012): 547-551.

45. Mukhram MA., *et al*. "Anti-fertility effect of flower extracts of *Tabernaemontana divaricata* in rats". *Chinese Journal of Natural Medicines* 10.1 (2012): 58-62.

46. Jain S., *et al*. "In vivo anti-inflammatory activity of *Tabernaemontana divaricata* leaf extract on male albino mice". *Chinese Journal of Natural Medicines* 11.5 (2013): 472-476.

47. Singh B., *et al*. "Antimicrobial, Antineoplastic and Cytotoxic Activities of Indole Alkaloids from *Tabernaemontana divaricata* (L.) R. Br". *Current Pharmaceutical Analysis* 7 (2011): 125-132.

48. Khan MSA. "Gastroprotective Effect of *Tabernaemontana divaricata* (Linn.) R.Br. Flower Methanolic Extract in Wistar Rats". *British Journal of Pharmaceutical Research* 1.3 (2011): 88-98.

49. Kam TS., *et al*. "Conodiparines A-D, new bisindole from *Tabernaemontana*. Reversal of vincristine-resistance with cultured cells". *Bioorganic and Medicinal Chemistry Letters* 8 (1998): 1693-1696.

50. Kanthlal SK., *et al*. "Amelioration of oxidative stress by *Tabernaemontana divaricata* on alloxan-induced diabetic rats". *Annals of Science Life* 33.4 (2014): 222-228.

51. Kojima I and Umezawa K. "Conophylline: A novel differentiation inducer for pancreatic β cells". *International Journal of Biochemistry and Cell Biology* 38.5-6 (2006): 923-930.

52. Devaraj P., *et al*. "Synthesis and characterisation of silver nanoparticles using *Tabernaemontana divaricata* and its cytotoxic activity against MCF7 cell line". *International Journal of Pharmacy and Pharmaceutical Sciences* 6.8 (2014): 86-90.

53. Anbukkarasi M., *et al*. "Comparison of the efficacy of a *Tabernaemontana divaricata* extract and of biosynthesized silver nanoparticles in preventing cataract formation in an in-vivo system of selenite-induced cataractogenesis". *Biocatalysis and Agricultural Biotechnology* (2020).