

## Pharmacological insights into *Ipomoea staphylina*: Therapeutic activities and the isolated bioactive metabolic compounds

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## MINI - REVIEW

## Pharmacological insights into *Ipomoea staphylina*: Therapeutic activities and the isolated bioactive metabolic compounds

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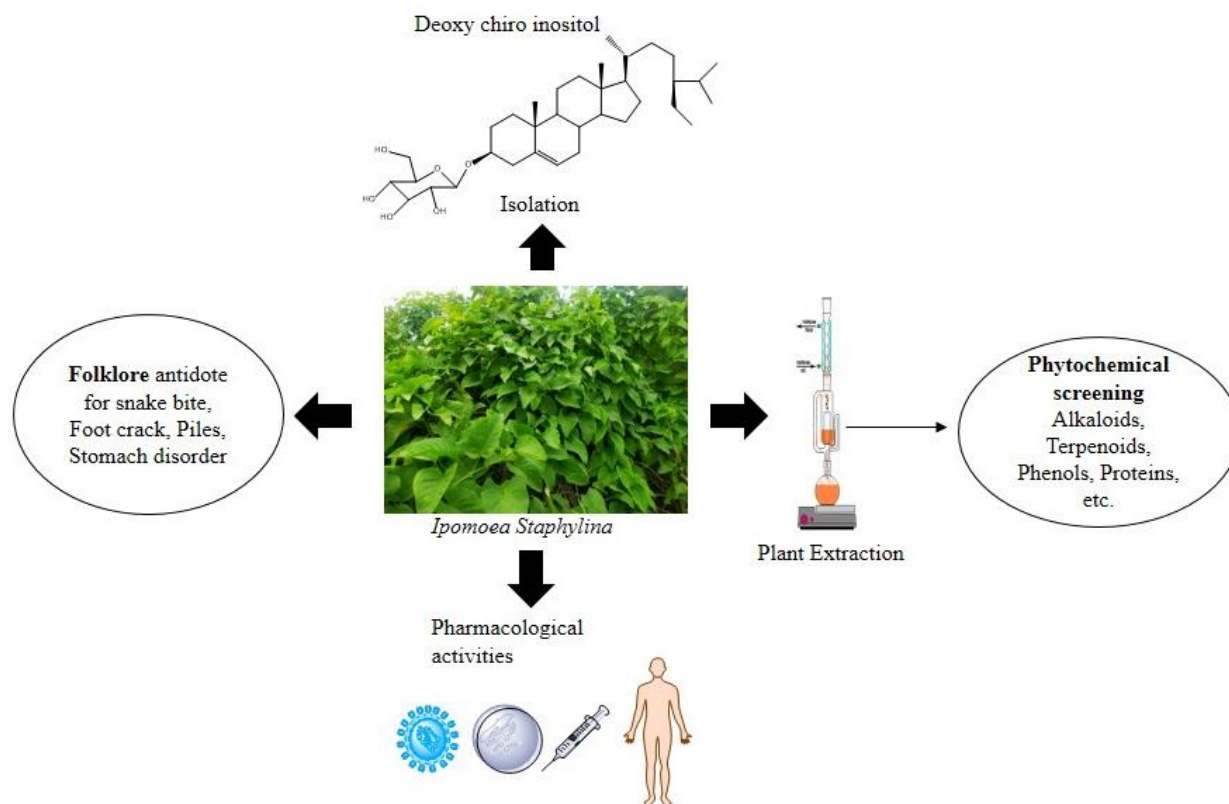
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### 6 ABSTRACT

This review comprehensively explores *I. staphylina*'s traditional uses, diverse applications, and pharmacological activities. Extensively used in traditional medicine, this plant addresses a range of ailments, including stomach disorders, respiratory issues, and rheumatism. Research has highlighted its significant antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anthelmintic, and analgesic properties. Notably, its antiulcer activity highlights its potential as a novel antiulcer agent, while hepatoprotective and nephroprotective effects suggest therapeutic applications in liver and kidney disorders. Studies on its anti-diabetic potential show significant reductions in blood glucose levels and positive impacts on biochemical markers. The plant's anti-mutagenic activity against base-pair mutations expands its potential applications. The review also discusses the isolation and pharmacological applications of pure compounds identified through LC-MS and NMR analyses. This review identifies *I. staphylina* as a promising source of bioactive compounds with therapeutic potential, emphasizing the need for further research to isolate and characterize its active constituents.

**Keywords:** *Ipomoea staphylina*; Biological activities; Plant extract; Isolation

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21 **Graphical abstract****ABBREVIATIONS:**

- 24 MTT - Microculture Tetrazolium; TNF- $\alpha$  - Tumor necrosis factor; LPS - Lipopolysaccharide; COX - Cyclooxygenase; DPPH - 2,2-Diphenyl-1-picrylhydrazyl; NBT - Nitro Blue Tetrazolium; ABTS- 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid; TBARS - Thiobarbituric acid reactive  
 27 substance; SGPT- Serum glutamic pyruvic transaminase; SGOT- Serum glutamic oxaloacetic transaminase; GSH- Glutathione; SOD- Superoxide dismutase; GPx - Glutathione peroxidase; LPO - Lipid peroxidase; CAT - Catalase; STZ - Streptozotocin; AST - aspartate  
 30 aminotransferase; ALT - alanine aminotransferase; ALP - Alkaline phosphatase; NMR - Nuclear magnetic resonance; LCMS - Liquid chromatography - mass spectrometry; HRTEM - High-Resolution Transmission Electron Microscopy;

## 33 INTRODUCTION

Plants have long been relied upon by humanity for their medicinal properties and their diverse applications in various aspects of daily life, including food, clothing, flavors, dyes, and more [1].  
36 Traditional herbal medicine, with its roots dating back centuries, forms the basis of modern healthcare systems. Interestingly, even in developed nations such as China, India, and Japan, traditional medicine continues to be practiced [2]. Despite the prevalence of synthetic  
39 alternatives in today's digital age, many people remain cautious about the side effects and chemical constituents associated with synthetic medicines. Synthetic drugs, including antibiotics, are known to induce adverse effects such as nausea, vomiting, and photosensitivity  
42 [3]. Consequently, there is a growing interest in natural medicines and compounds that offer potential benefits with fewer side effects and a more organic impact on the human body [4]. Natural products, which can be readily integrated into our daily diet, are easily absorbed and  
45 often preferred. According to the World Health Organization, approximately 21,000 plant species worldwide have been utilized for medicinal purposes [5]. Remarkably, over 60% to 75% of cancer and infectious disease drugs have been derived from natural sources [6, 7]. Plant-  
48 based drugs have therefore gained significant traction in contemporary drug design and development [8, 9].

One notable herb with significant therapeutic potential is *Ipomoea staphylina*, which holds a  
51 prominent place in traditional medicine [10, 11]. Traditionally, *I. staphylina* has been employed to address various disorders [10, 11]. Its distribution spans regions in India, China, and Sri Lanka, as well as numerous parts of Asia and South Asia [12]. Recent research has highlighted  
54 the antibacterial, anti-diabetic, anti-inflammatory, anti-mutagenic, analgesic, and antioxidant properties of *I. staphylina* [3]. Abundantly found in wastelands and deciduous forests, *I. staphylina* is easily accessible. It possesses a rich composition of phytochemical constituents,  
57 including alkaloids, flavonoids, phenolics, proteins, carbohydrates, glycosides, and saponins [3, 13, 14]. In local languages, *I. staphylina* is referred to as "Oonan kodi" or "Onan kodi" in Tamil, "Ugina kodi" or "Unang kodi" in Kannada, and "Sunang kodi" in Irula, while it is known  
60 as "Morning clustered glory" in English [9, 14]. Through this literature review, we aim to provide an in-depth exploration of the ethnobotanical and pharmacological properties of *I. staphylina*, shedding light on its potential benefits in healthcare and therapeutic applications. The clear  
63 image represents the different plant parts of the *I. staphylina* depicted in Figs. 1 and 2.



Fig. 1. (a, b) Leaves of the *I. Staphylina*, (c) Flower of the *I. staphylina*.

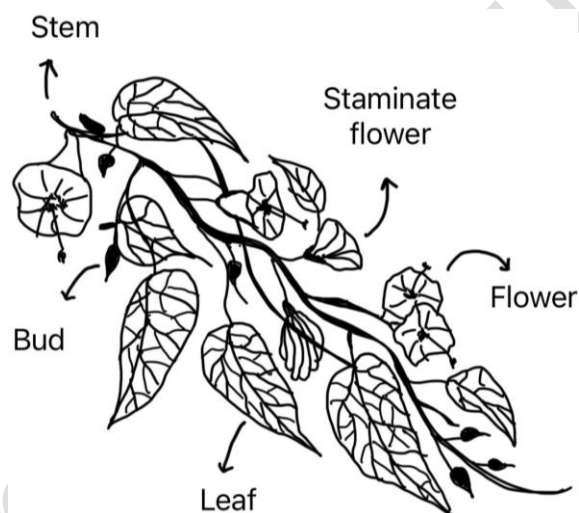


Fig. 2. A formal botanical sketch of *I. staphylina*.

### **Traditional uses**

*I. staphylina* has been used for many health issues like stomach disorders, inflammation, purgation, pain, and also in rheumatism [15]. Traditionally Dharmapuri (Tamil Nadu) village folk people used stem raw extract for stomach disorders and respiratory disorders. Gingee Hills villagers used leaf latex for foot crack [7]. Tribes of Irulas and Palliyars have used *I. staphylina* roots as an anti-dote (snake bite) [8]. Karandamalai villagers used leave decoction for stomach disorders [16]. Chenchus tribes treated leave extract for piles (Andhra Pradesh) [17].

## METHODS

### 75 **Search strategy**

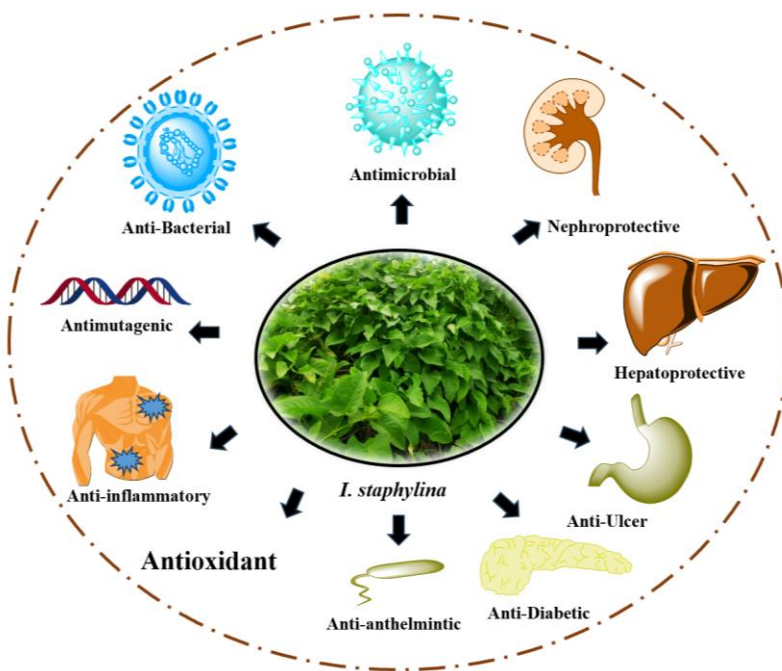
In this review, we systematically compiled data from multiple scientific databases, including Web of Science, Science Direct, Google Scholar, and MEDLINE, focusing on *I. staphyлина*.

78 Topics covered include plant extraction, isolation, characterization, biological activity, and nanoparticle synthesis. Our analysis integrates findings from both in vitro and in vivo studies, with data collected from the inception of these databases up to March 2024. Publications in  
81 languages other than English, as well as conference abstracts and dissertations, were excluded from the review.

### **Study selection**

84 Publications in languages other than English, along with conference abstracts and dissertations, were excluded from consideration. The initial search yielded 60 studies, from which duplicate entries were removed. The screening process involved evaluating titles,  
87 abstracts, and full texts according to predefined inclusion criteria, resulting in the final inclusion of 44 relevant studies in the review.

### **Pharmacological activities**



90 Fig. 3. Pharmacological applications of *I. staphyлина*

*I. staphylina* has been reported for various pharmacological activities in different solvents and methods shown in Table 1 and Fig. 3.

93 Table 1. Biological activities of *I. staphylina*

Pharmacological effects	Plant part(s)	Solvent of the crude extract	References
Antioxidant, cytotoxic	Whole plant	Ethanol	Padmashree M.S <i>et al.</i> , (2018)
Hepatoprotective and antioxidant (in vivo)	Leaves	Aqueous	Jayadevi R <i>et al.</i> , (2019)
Hepatoprotective and nephroprotective	Leaves	Hydroalcoholic	Bag A.K <i>et al.</i> , (2013)
Cytotoxic, Antimicrobial and anti-inflammatory	Stems, Crude latex	Water Ethanol n-Butanol	Narra P <i>et al.</i> , (2014)
Anti-mutagenic	Leaves	Hydroalcoholic	Banerjee A <i>et al.</i> , (2020)
Anti-ulcer	Whole plant	Hydroalcoholic	Banerjee A <i>et al.</i> , (2014)
Anti-anthelmintic	Leaves	Aqueous	Gosh S <i>et al.</i> , (2013)
Adulticidal Larvicidal	Leaves	Hexane Ethyl acetate Acetone Methanol	Santhoskumar T <i>et al.</i> , (2011)
Anti-inflammatory	Leaves	Ethanol	Firdous <i>et al.</i> , (2012)
Anti-diabetic	Leaves	Ethanol	Firdous <i>et al.</i> , (2014)
Anti-diabetic type 2	Leaves	Ethanol	Firdous <i>et al.</i> , (2016)
Anti-diabetic	Whole plant & leaf	Ethanol	Shobana D <i>et al.</i> , (2021)
Antioxidant, Superoxide Anti-diabetic Anti-inflammatory (LOX-5)	Whole plant	Hydroalcoholic	Reddy D.P <i>et al.</i> , (2012)
Analgesic	Leaves	Hydroalcoholic	Gosh S <i>et al.</i> , (2014)

### Antimicrobial activity

In a research study conducted by Narra *et al.*, the antimicrobial potential of the crude latex obtained from the stems of *I. staphylina* was investigated. The study aimed to evaluate its activity against *Staphylococcus aureus* and *E. coli* bacteria, two common pathogens known to cause various infections. Different concentrations of the extract were utilized to determine its efficacy in inhibiting the growth of these bacteria [13]. The study demonstrated that the ethanol extract of *I. staphylina* exhibited significant inhibitory activity against *S. aureus* and *E. coli*, indicating its potential as a source of novel antimicrobial agents. To evaluate the safety profile of the extract, an MTT assay was conducted using J774.2 cells, a standard model for assessing cell viability and cytotoxicity of test compounds. The assay results offered crucial insights into

the extract's effects on cell viability, supporting its potential therapeutic application. Overall, the study by Narra *et al* sheds light on the antimicrobial activity of the crude latex from *Ipomoea staphylina* stems and highlights its potential as a source of natural compounds with antimicrobial properties [13]. Given its excellent antimicrobial activity, formulations such as antimicrobial ointment or cream could be developed from this plant.

### **Anti-inflammatory activity**

Firdous *et al.* (2012) investigated the anti-inflammatory properties of the ethanolic leaf extract of *I. staphylina*. The study evaluated both the ethanolic extract and its ethyl acetate and n-butanol fractions through in vivo and in vitro models. The fractions displayed significant anti-inflammatory activity in carrageenan-induced paw edema. Additionally, when administered orally at a dose of 200 mg/kg, the extract and its fractions significantly reduced granuloma formation in the cotton pellet-induced granuloma model ( $P > 0.001$ ). The study also examined the inhibition of TNF- $\alpha$  in LPS-activated RAW 264.7 cells, where the ethanol, n-butanol, and ethyl acetate fractions showed notable inhibitory effects. Cell viability across different concentrations was further assessed using the MTT assay [15].

Narra *et al.* (2014) focused on the stem ethanol extract of *I. staphylina* to evaluate its anti-inflammatory properties through COX activity assay. The COX assay was performed using a specific chemiluminescent substrate to measure the activity. The latex sample (approximately 20  $\mu$ l) was added and incubated for 2 hours, followed by the addition of 50  $\mu$ l of the chemiluminescent substrate to determine the activity. The ethanolic extract demonstrated potent inhibitory activity against COX [13].

In a study by Reddy *et al.* (2012), the entire plant of *I. staphylina* was evaluated for its anti-inflammatory activities using a modified version of the techniques. The methanolic and hydroalcoholic extracts of *I. staphylina* were assessed for their ability to inhibit LOX-5 enzyme. The percentage of inhibition was determined, and the extracts of *I. staphylina* exhibited 50% lower inhibitory percentages compared to standard drugs [14]. The reported anti-inflammatory properties of *I. staphylina*, both in vivo and in vitro, highlight its importance in inflammation management in medical situations. Its potent activity against important inflammatory enzymes such as COX and LOX-5 makes it an attractive candidate for formulations targeting inflammatory disorders [13-15]. Given its shown efficacy and minimal toxicity profile, *I. staphylina* is a promising candidate for the development of anti-inflammatory formulations, such



135 as gel or tablet formulations. These compositions with significant anti-inflammatory activities  
136 have the potential to provide effective treatment while minimizing side effects, addressing a  
137 critical demand in inflammatory therapy.

### 138 **Anti-oxidant and free radical scavenging activity**

139 Reddy *et al.* (2012) conducted a study to investigate the antioxidant activity of methanolic and  
140 hydroalcoholic extracts derived from the entire plant of *I. staphylina*. The DPPH radical  
141 scavenging method was employed to assess the free radical activity. Various concentrations  
142 (25, 50, 75, and 100 µg/ml) of the fractions were prepared, and their absorbance at 517 nm  
143 was measured to determine the activity. The results revealed that the hydroalcoholic stem  
144 extract and methanolic leaf extract exhibited noteworthy antioxidant activity compared to other  
145 extracts [14].

146 The superoxide radical scavenging activity of the methanolic leaf extract of *I. staphylina* was  
147 investigated using the Nitro Blue Tetrazolium (NBT) riboflavin photoreduction method, following  
148 the methodology established by Mccord and Fridovich. Reddy *et al.*, testified that the  
149 hydroalcoholic extract derived from the stem (IC<sub>50</sub> = 15.54 µg/ml) and leaf (IC<sub>50</sub> = 16.02 µg/ml)  
150 of *I. staphylina* demonstrated remarkable efficacy compared to other extracts evaluated in the  
151 study [14].

152 Furthermore, the antioxidant potential of the ethanolic leaf extract of *I. staphylina* was assessed  
153 using a comprehensive range of assays. These included measurements of superoxide radical  
154 scavenging, hydroxyl radical scavenging, DPPH assay, ABTS assay, and metal chelating assay.  
155 The extract was subjected to various concentrations, and the IC<sub>50</sub> values were determined  
156 employing standard protocols. Furthermore, the study conducted by Shobana D *et al.*, in 2021  
157 investigated the antioxidant activity of the ethanolic leaf extract and whole plant extract of *I.*  
158 *staphylina* on streptozotocin-induced rats, yielding excellent results [14, 18]. The findings from  
159 the above-mentioned studies provided valuable insights into the antioxidant properties of *I.*  
160 *staphylina*, further enhancing its potential therapeutic applications [14]. The biochemical  
161 measurements in the rats were performed using the methods outlined in Table 2. These  
162 reported antioxidant activities suggest that extracts from *I. staphylina* can be formulated into  
163 antioxidant drugs.

Table 2. Methods used for biochemical measurements in diabetic-induced rats

Experiment	References
Thiobarbituric acid reactive substance (TBARS) and lipid peroxide (HP)	Jean <i>et al.</i> , (1992)
The measurement of glutathione in the tissue (GSH)	Beutler, (1984)
Superoxide dismutase (SOD)	Kakkar <i>et al.</i> , (1984)
Glutathione peroxidase (GPx)	Rotruck <i>et al.</i> , (1984)
Anti-oxidant (Vitamin C and Vitamin E)	Bakers <i>et al.</i> , (1980)

### 165 **Anti-diabetic activity**

In a study conducted by Shobana D *et al.* (2021), the ethanolic leaf extract and whole plant extract of *I. staphyлина* were investigated for their antioxidant, peroxide, and anti-diabetic activities. Male Wistar rats were used, and administration of Streptozotocin and diabetes was induced through intraperitoneal. The assessment of biochemical parameters was performed using established methods. The results demonstrated that *I. staphyлина* extracts pointedly reduced glucose levels in diabetic-induced rats and increased antioxidant levels compared to normal rats. Moreover, the extracts exhibited notable reductions in glutathione peroxidase, hyperoxide, and TBARS levels in diabetic rat tissues. Among the extracts, the ethanolic leaf extract of *I. staphyлина* showed the most promising results in diabetic-induced rats [18].

Firdous *et al.*, (2014) conducted a study to investigate the anti-diabetic activity of the ethanolic extract of *I. staphyлина* in Swiss albino mice induced with Streptozotocin. The extract was orally administered, and no acute toxicity or signs of toxicity were observed. The ethanolic extract notably reduced blood glucose levels. Biochemical analysis revealed significant decreases in serum creatinine blood urea, and blood urea nitrogen, levels upon treatment with the extract. Additionally, the ethanolic extract led to reductions in serum total protein and liver glycogen levels in streptozotocin-induced diabetic mice. The ethanol, n-butanol, and ethyl acetate extract fractions exhibited decreases in SGOT, ALP, and SGPT activities in diabetic mice. Furthermore, the ethanol extract and ethyl acetate fraction demonstrated enhancements in GPx, CAT, and SOD levels [19]. Biochemical analysis, as outlined in Table 3.

Table 3. *In vivo* biochemical measurements and methods [19].

Biochemicals	Assay and Methods
Serum glucose	Glucometer (Accu-Chek Active, India)
Serum total cholesterol, total triglyceride, LDL-c, VLDL-c and HDL-c	Standard enzymatic (Span Diagnostic, India)

SGOT, SGPT, serum ALP, total protein, serum urea, and creatinine	Standard enzymatic (span diagnostic, India)
Glycogen content in the liver	Spectrophotometric determination of glycogen with o-toluidine reagent
Lipid peroxidase (LPO), Superoxide dismutase (SOD), Catalase (CAT) and glutathione peroxidase (GPx).	10% ice-chilled potassium chloride solution

186 In a study by Firdous *et al.* (2016), the ethanol, n-butanol, and ethyl acetate extract fractions of  
 189 *I. staphyлина* were assessed for their anti-diabetic property. Alloxan-induced diabetic rats were  
 used in the study, and the extracts were administered orally. The extracts significantly reduced  
 the blood glucose levels.

Moreover, Firdous *et al.* (2016) examined the anti-diabetic effects of the ethanol, n-butanol, and  
 ethyl acetate leaf extract fractions of *I. staphyлина* against nicotinamide-streptozotocin-induced  
 192 type 2 diabetes. Wistar rats were employed in this study. The findings indicated a significant  
 reduction in blood glucose levels following treatment with the leaf extracts. Additionally, the  
 extracts led to reductions in biochemical markers such as SGOT, SGPT, and ALP. Histological  
 195 analysis of the liver, kidney, and pancreas, revealed improvements at the cellular level after 21  
 days of oral administration of *I. staphyлина* extract and its fractions in nicotinamide-  
 streptozotocin induced type 2 diabetic rats [20]. According to the studies mentioned above,  
 198 various parts of the *I. staphyлина* plant demonstrate remarkable antidiabetic activities effective  
 against both type 1 and type 2 diabetes [18-20]. Through oral administration, these plant parts  
 effectively reduce blood glucose levels. Consequently, the therapeutic potential of *I. staphyлина*  
 201 in the management of diabetes is evident, suggesting its utility in the development of diabetic  
 medications. This could include the formulation of oral tablets or syrups tailored to regulate  
 blood sugar levels and alleviate symptoms associated with diabetes.

#### 204 ***Anthelmintic activity***

In research conducted by Gosh S *et al.* (2013), the distilled water extract of *I. staphyлина* was  
 evaluated for its anthelmintic ability. The extract was tested against *Perionyx excavates*  
 207 earthworm at three different concentrations (25, 50, and 100 mg/ml). A standard drug,  
 Piperazine citrate, was used for comparison. The findings indicated that the *I. staphyлина* plant  
 extract did not exhibit any anthelmintic activity on earthworms, as evidenced by the results  
 210 obtained [21].

### **Anti-ulcer activity**

213 Banerjee A *et al.* (2015) conducted a scientific investigation to estimate the anti-ulcer activity of  
a water and ethanolic (3:7) extract derived from *I. staphylina*. The study employed dried  
powdered samples of the entire plant to assess its efficacy. Two well-established experimental  
216 models, namely pyloric ligation and ethanol-induced gastric ulcer methods, were employed to  
simulate ulcer conditions. Oral administration of the *I. staphylina* plant extract was carried out  
in rats, and the acid index, a key indicator of ulcer severity, was determined using the standard  
drug Omeprazole for reference. The findings of the study revealed that the *I. staphylina* extract  
219 exhibited significant anti-ulcer properties, as evidenced by a remarkable reduction in acid pH  
levels. This indicates the plant's potential as a promising candidate for the development of novel  
anti-ulcer agents [22].

### **Hepatoprotectivity and Nephroprotectivity**

222 Bag A.K *et al.* (2013) conducted a study to assess the hepatoprotective and nephroprotective  
properties of a hydroalcoholic leaf extract from *I. staphylina*. To assess the nephroprotective  
225 and hepatoprotective activity, Wistar albino rats were induced with gentamicin and CCl<sub>4</sub>,  
respectively. The administration of the plant extract was done orally, and no signs of toxicity  
were observed.

228 For the evaluation of hepatoprotective activity induced by CCl<sub>4</sub>, the levels of AST, ALP, ALT,  
and total bilirubin were measured. The *I. staphylina* plant extract demonstrated a significant  
decrease in the levels of AST, ALP, ALT, and total bilirubin, indicating its potential  
231 hepatoprotective effects [23].

Furthermore, Jayadevi *et al.* (2019) reported the hepatoprotective activity of an aqueous leaf  
extract from *I. staphylina*. In a study conducted on CCl<sub>4</sub>-induced Wistar rats, the extract showed  
234 a reduction in liver enzymes such as SGOT, SGPT, ALP, and bilirubin. It is important to note  
that both studies highlight the protective properties of *I. staphylina* extracts on liver and kidney  
functions, suggesting their potential therapeutic applications in the treatment of hepatic and  
237 renal disorders [24].

### **Analgesic activity**

Ghosh *et al.*, (2014) conducted a study to assess the analgesic activity of the hydroalcoholic  
240 extract of *I. staphylina*. The evaluation was performed using established methods including

abdominal writhing, formalin-induced paw licking, and the Eddy's hot plate test in Swiss albino mice. As reference standards, Acetylsalicylic acid, Pentazocine, and Diclofenac were utilized.

243 The administration of the hydroalcoholic extract of *I. staphylina* at a dose of 200 mg/kg resulted in a significant analgesic effect. The observed activity suggests the potential of the extract as an analgesic agent, comparable to the standard drugs used in the study [25]. This research  
246 approach provides valuable insights into the analgesic properties of the hydroalcoholic extract of *I. staphylina*, indicating its potential for further exploration and development as a natural analgesic alternative

#### 249 **Adulticidal activity and Larvicidal activity**

In research conducted by Santoshkumar *et al.* (2011), the inhibitory properties of dried leaf extracts of *I. staphylina* were investigated. Various solvents such as ethyl acetate, hexane,  
252 methanol, and acetone were utilized for the extraction process. The evaluation focused on the inhibition of adults of *H. bispinosa*, the hematophagous fly *H. maculata*, and the *instar larvae* of the malaria vector *A. subpictus* [26].

255 The results demonstrated that the leaf extracts of *I. staphylina* exhibited significant inhibitory activity against these targeted organisms. This suggests the presence of bioactive compounds within the plant that have the potential to act as effective agents against these medically  
258 important vectors.

The findings from this scientific investigation provide valuable insights into the potential of *I. staphylina* as a natural source for developing novel insecticidal agents. Further research and  
261 exploration of the specific bioactive constituents responsible for the observed inhibitory effects are warranted for the development of effective vector control strategies.

#### **Anti-mutagenic activity**

264 Banerjee *et al.* (2020) performed research to investigate the anti-mutagenic activity of the hydroalcoholic (ethanol 7:3 distilled water) leaf extract of *I. staphylina* against base-pair mutations induced by 2-Nitrofluorene. Two nonvirulent strains of *S. typhi* were used to evaluate  
267 this activity, namely TA1535 & TA1538. Two different concentrations of the plant extract (250 and 500 µg/ml) were employed along with Cyclophosphamide (50 µg/ml) as a reference.

The assessment of anti-mutagenic activity was carried out using the Ames method. The results  
270 obtained from the study indicated that *I. staphylina* exhibits anti-mutagenic activity, and the

extent of this activity depends on the dosage of the plant extract [27]. These findings shed light on the potential of *I. staphylina* as a source of anti-mutagenic compounds. Further research is warranted to identify and isolate the specific constituents responsible for this activity, and to explore their potential for therapeutic applications in mutagenesis-related disorders.

### Phytochemical profile

Secondary metabolites are chemical compounds responsible for the biological properties of plants or herbs [31-33]. Various parts of *I. staphylina* have been reported to contain important phytochemicals, including alkaloids, terpenoids, tannins, flavonoids, carbohydrates, sterols, saponins, and phenols [13-14,18-24]. These secondary metabolites contribute significantly to enhancing the medicinal properties of *I. staphylina* [13-18]. Also, these phytochemicals exert crucial roles in human health, functioning as antioxidants, antibacterial, antifungal, anti-inflammatory, anti-allergic, antispasmodic, chemopreventive, hepatoprotective, hypolipidemic, neuroprotective, hypotensive agents. They contribute to preventing aging, diabetes, osteoporosis, cancer, and heart diseases [31-36], while inducing apoptosis, acting as diuretics and CNS stimulants, and providing analgesic effects. Furthermore, they shield against UVB-induced carcinogenesis, modulate the immune system, and possess carminative properties [31-36].

### Isolated compounds

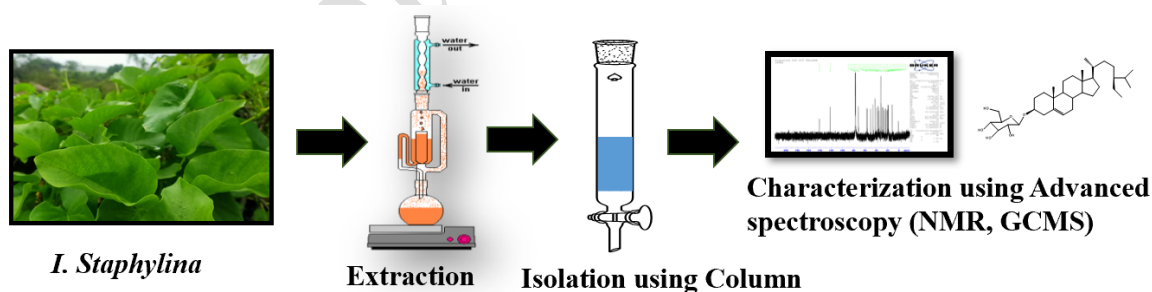


Fig. 4. Isolation of chemical compound from plant extracts

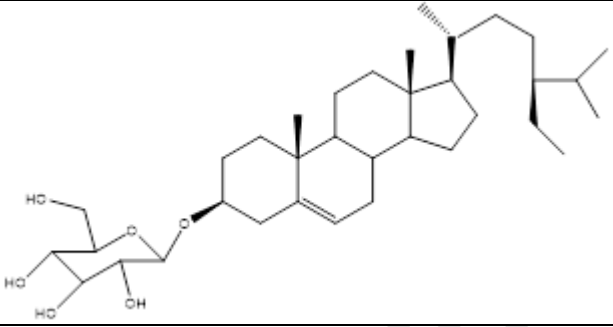
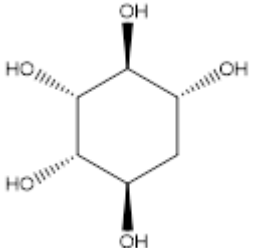
In a study conducted by Reddy *et al.* (2012), two pure compounds were isolated from the methanolic leaf extract of *I. staphylina*. The pictorial illustration of the isolation is represented in Fig. 4. The identification and confirmation of these compounds were carried out using LC-MS and NMR analysis. Detailed information and characteristics of these isolated compounds are provided in Table 4. The utilization of analytical techniques such as LC-MS and NMR ensures

the accurate identification and structural elucidation of the compounds, providing valuable  
insights into their chemical composition and potential biological activities [14]. The isolation of  
these pure compounds from *I. staphylina* adds to the knowledge of its phytochemical profile,  
and further investigation is warranted to determine their specific biological properties and  
potential applications in various fields, such as medicine, and pharmacology. The isolated  
compound **1**, sitosteryl-3-O- $\beta$ -D-glucoside, has been reported to exhibit various biological  
activities, including analgesic and anti-inflammatory effects [28]. The parent moiety of sitosteryl-  
3-O- $\beta$ -D-glucoside, beta-sitosterol, possesses a wide range of biological activities, such as  
antimicrobial, anticancer, antidiabetic, antioxidant, angiogenic, immunomodulatory, anti-  
inflammatory, and antinociceptive properties, without significant toxicity [29, 30].

In 2018, Padmashree *et al.* analyzed the ethanolic extract derived from *I. staphylina* using GC-  
MS. This analysis revealed the presence of several significant bioactive compounds. Among  
these compounds were 2-furanmethanol, 1,2-benzenediol, 2-methoxy-4-vinylphenol,  
tocopherol, beta-sitosterol, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, and 4-((1E)-  
3-hydroxy-1-propenyl)-2-methoxyphenol, alongside 2,6,10,14,18,22-tetracosahexaene,  
2,6,10,15,19,23-hexamethyl-, (all-E), and hexadecanoic acid. Notably, these compounds are  
known for their remarkable antioxidant activities [36]. This research underscores the potential  
of *I. staphylina* as a valuable source of bioactive compounds with antioxidant properties [36].  
Additionally, minor percentages of compounds such as 3-(4-hydroxyphenyl)-, vitamin E,  
9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)-3,7,11,15-tetramethyl-2-hexadecen-1-ol; 4-  
((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol, caryophyllene, 4H-pyran-4-one, 2,3-dihydro-  
3,5-dihydroxy-6-methyl-, cyclopentane, 1-acetyl-1,2-epoxy-2-propenoic acid,  $\gamma$ -tocopherol,  
phytol,  $\alpha$ -caryophyllene, and 2-methoxy-4-vinylphenol have been identified [36].

The plant species harbors numerous active compounds, predominantly phenols,  
carbohydrates, and sterol-like constituents within the extract of *I. staphylina* [36, 37]. These  
compounds, acting synergistically, contribute significantly to the plant's medicinal properties,  
such as antioxidant, anthelmintic, anti-inflammatory, anti-diabetic, antimicrobial, and analgesic  
activities [31, 32, 36, 37]. Additionally, the isolated compound **2**, deoxychiro-inositol, has been  
reported to exhibit insulin-like actions [38].

Table 4. Isolated compounds of *I. staphylina* [14].

Compounds	Extract	Structure
Sitosteryl-3-O- $\beta$ -D-glucoside	Ethanol extract of <i>I. staphylina</i>	
Deoxy chiro inositol	Ethanol extract of <i>I. staphylina</i>	

### Nanoparticle synthesis

327 Over the last few decades, there has been a rise in the fabrication of therapeutic and diagnostic  
agents based on nanoparticles for various medical uses, with plants and herbs emerging as  
promising sources for nanoparticle synthesis [39, 40]. Various biological sources harbour the  
330 potential to yield a wide array of nanoparticles, each characterized by unique properties,  
shapes, and sizes [41, 42]. In the past few years, much research has been done in the  
Nanomaterials field using the *I. staphylina* plant [39-42]. In 2020, Pugazhendhi synthesized  
333 silver nanoparticles utilizing *I. staphylina* leaf extract, employing green chemistry techniques  
[43]. The synthesized nanoparticles underwent characterization via UV-Vis spectroscopy,  
FTIR, HRTEM, and XRD. The average particle size of the synthesized silver nanoparticles,  
336 determined using Scherrer's formula, was found to be approximately 20 nm, a value  
corroborated by HRTEM analysis (22 nm) [43].

In a recent study by Lakshmanan N *et al.* in 2024, silver and CuO nanoparticles were  
339 synthesized using the ethanolic extract of *I. staphylina* for larvicidal activity [44]. Employing  
environmentally sustainable green chemistry methodologies, the researchers validated the  
nanoparticle structure and size through XRD analysis, while field-emission scanning electron  
342 microscopy (FE-SEM) revealed precise nanostructures. Elemental composition was elucidated  
via energy-dispersive X-ray (EDX) analysis, and UV-vis spectroscopy provided bandgap energy  
values (3.15 eV for silver, 1.2 eV for CuO nanoparticles). These nanoparticles displayed



345 potential larvicidal activity, with CuO nanoparticles demonstrating superior LC<sub>50</sub> and LC<sub>90</sub>  
values compared to silver nanoparticles. Furthermore, the developmental toxicity of CuO and  
348 Ag NPs was assessed in zebrafish embryos as part of non-target eco-toxicological  
investigations conducted in a standard laboratory setting. These findings highlight the potential  
of these nanoparticles as highly effective and ecologically friendly natural insecticides, providing  
cost-effectiveness and ecological benefits [44].

## 351 CONCLUSION

The documented pharmacological properties of *I. staphylina* substantiate its medicinal uses,  
further validating its traditional significance based on ethnobotanical knowledge. This plant has  
354 been reported to possess a range of biological activities, including antibacterial, anti-  
inflammatory, anti-mutagenic, hepatoprotective, nephroprotective, antioxidant, anti-ulcer, and  
anti-diabetic, anthelmintic, and analgesic effects. These diverse therapeutic applications can  
357 be attributed to the presence of various bioactive phytoconstituents within the plant. The  
findings from reported studies on *I. staphylina* underscore the importance of this botanical  
resource in healthcare. This article aims to provide comprehensive pharmacological and  
360 ethnobotanical information about *I. staphylina*, thereby facilitating further exploration and  
understanding of the plant's potential benefits. By shedding light on its pharmacological  
attributes and traditional uses, this research contributes to the holistic appreciation and  
363 utilization of *I. staphylina* as a valuable resource in the field of medicine.

## Declaration of interest

The authors declare that they have no known competing financial interests or personal  
366 relationships that could have appeared to influence the work reported in this paper.

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## AUTHOR CONTRIBUTIONS

Mr. Lakshmanan Narayanan collected the plant details and wrote the paper. This review paper  
372 was supervised and edited by Dr. Suseem S.R. All authors read and approved the final  
manuscript.

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