



Original article

Analysis of the chemical composition of root essential oil from Indian sarsaparilla (*Hemidesmus indicus*) and its application as an ecofriendly insecticide and pharmacological agent

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ARTICLE INFO

Article history:

Received 11 July 2021

Revised 9 August 2021

Accepted 9 August 2021

Available online xxx

Keyword:

Hemidesmus indicus

Root essential oil

Fumigant activity

Repellent effect

Biopesticidal activity

Sitophilus oryzae

Callosobruchus maculatus

Antioxidant activity

Anti-diabetic activity

ABSTRACT

The Indian sarsaparilla (*Hemidesmus indicus*) is a commonly used plant in Indian traditional medicine of Ayurveda for the preparation of various non-alcoholic beverages. However, limited studies are available on the essential oil of *H. indicus* roots (HRO); therefore, the study evaluated the antioxidant, anti-inflammatory and antidiabetic activities of *H. indicus* root essential oil as well as insecticide potential against the common pests of stored food materials (*Sitophilus oryzae*, *Callosobruchus maculatus* and *Tribolium castaneum*). The repellent efficacy of HRO was found to be high against *S. oryzae* ($8.21 \pm 0.55 \mu\text{g/mL}$). Likewise, the fumigant potential was also observed for HRO against these pests; the higher activities were observed against *S. oryzae* and *C. maculatus* (32.46 ± 1.42 and $35.18 \pm 1.62 \mu\text{g/L}$). Besides, the essential oil was also found to be active as a contact poison, however, against all the three pests, the toxicity was above $100 \mu\text{g/mm}^3$, being the highest against *C. maculatus* ($122.8 \pm 3.57 \mu\text{g/mm}^3$). To analyze the possible effect of the essential oil on grains, the different grains were allowed to germinate and compared to that of normal; thus, the non-toxic nature of HRO against the stored products is also confirmed. The essential oil shown to have DPPH hydrogen peroxide and ABTS radical scavenging, nitric oxide scavenging potential, and inhibition of lipoxigenase, alpha-amylase and alpha-glucosidase. Overall, the present study concludes that the *H. indicus* may be a suitable repellent and fumigant agent against different pests of stored products and a possible antioxidant, anti-inflammatory, and anti-diabetic agent.

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1. Introduction

Pests are animals that are having harmful effects directly or indirectly on crops, livestock, or humans. Among the different pests, insects are thought to be one of the most important organisms with pest status. Pests can cause serious loss to the economy of a country by reducing the productivity of various crops (Hernandez Nopsa et al., 2015). Apart from these, the particular

group of insect pests that feed on the stored food products are more damaging, as they damage the stored food materials (Kumar and Kalita, 2017). Cereals and pulses are the important products that supply food materials to the whole world and the pests acting on them are therefore causing a serious threat to food safety (Yaseen et al., 2019). The most common pests of the stored grains include *Sitophilus oryzae*, *S. zeamais*, *Tribolium* sp., and *Callosobruchus* sp that feed on grains and other pulses. Fumigation with various pesticides is the currently employed strategy for the management of these pests; the commonly used pesticides include phosphine, sulfuryl fluoride, and pyrethrins (Huang and Subramanyam, 2005; Jagadeesan et al., 2018). However, the use of fumigant pesticides are known to cause deleterious effects to humans also; the exposure can induce toxic effects on the central nervous system, various types of cancers and acute poisoning often results in life-threatening conditions (Alavanja et al., 2014; Park

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Peer review under responsibility of King Saud University.



et al., 2020; Van de Sijpe et al., 2020). This intensified the requirement for new pesticide compounds that are having significant repellency and fumigant potential to the different pests and are less toxic to humans.

Plant-derived essential oils are emerging as key pesticide molecules that are having less impact on the environment as well as on human/animal/plant health (Al Dawsari Mona, 2020; Zaka et al., 2019). Among the different essential oils produced, those produced from edible plants or plant parts are in the limelight of becoming an eco-friendly biocide. Apocynaceae family is one of the widely studied family for their biological, toxicological, pharmacological, ethno-medicinal, and botanical values (Bhadane et al., 2018). Several plants belonging to the group are evolved as promising insecticidal, larvicidal and antimicrobial drug candidates (Dhileepan, 2014; Panneerselvam et al., 2013). Among these plants, the Indian sarsaparilla (*Hemidesmus indicus*) is utilized as an aromatic agent in a variety of food and beverages (Nandy et al., 2020). The plant is reported to be beneficial against a variety of disease conditions by virtue of its antioxidant and anti-inflammatory potentials (Desai et al., 2017; Kundu and Mitra, 2013). Further, the plant is found to reduce secondary diabetic complications by inhibiting the polyol pathway enzyme aldose reductase (Haroon et al., 2021). Anticancer properties are also attributed to the plant against colon (Turrini et al., 2019), breast (Bansod et al., 2021) and lung cancer cells (Pathiranage et al., 2020). However, limited studies are available on the essential oil of *H. indicus*; a previous study has identified the bioactive component 2-hydroxy-4-methoxy benzaldehyde (Jirovetz et al., 2002). It is, therefore, necessary to evaluate the potential of the essential oil as a possible biocide against different pests of stored products including *Sitophilus oryzae*, *Callosobruchus maculatus* and *Tribolium castaneum*. Further, the essential oil was also evaluated for their anti-radical effects, anti-diabetic effects, and toxic/adverse effects in terms of the germination potential of grains.

2. Materials and methods

2.1. Plant materials collection and essential oil extraction

Hemidesmus indicus roots were collected from naturally growing areas in the Calicut district, Kerala, India. The roots were then cleaned to remove soil, washed in cold water and powdered. The essential oil was extracted by steam distillation in three independent isolations; the yield and composition were compared. The preparation was according to the modified methods of Nagarajan et al. (2001); briefly, about 250 g of the freshly powdered roots of *H. indicus* was weighed and transferred to a steam distiller (6 h). The organic essential oil layer was then isolated and concentrated before storage at -20°C .

2.2. Chemical composition analysis by GC/MS

Chemical composition was analyzed using a Varian CP 3800 GC/MS system with a column size of $30\text{ m} \times 0.25\text{ mm}$ inner diameter and a thickness of $0.25\text{ }\mu\text{m}$. The analysis conditions were as follows; the oven temperature of 50°C (120 s), temperature rise ($2^{\circ}\text{C}/\text{min}$ till 250°C), the temperature of injection port and detector was 250°C . The helium was used as the carrier gas at a flow rate of $1\text{ mL}/\text{min}$. The compound identification was carried out by comparing the retention index (RI) with the NIST library.

2.3. Radical scavenging and anti-inflammatory potentials

The DPPH (2,2-diphenyl-1-picrylhydrazyl) and 2,2'-Azino-bis(3-ethyl benzthiazoline-6-sulfonic acid (ABTS⁺) inhibition assays

were conducted as per the methods described by Alara et al. (2019). Hydrogen peroxide scavenging potential was estimated according to the protocols mentioned in Saeed et al. (2021). The anti-inflammatory potential was estimated as nitric oxide scavenging (Mahomoodally et al., 2019) and lipoxygenase inhibition (Alzarea et al., 2021) assays according to the standard protocols.

2.4. Inhibition of alpha-amylase and alpha-glucosidase inhibition

2.4.1. α -Amylase inhibition activity

Different concentrations (0–100 $\mu\text{g}/\text{mL}$) of essential oil were mixed with 400 $\mu\text{g}/\text{mL}$ of porcine α -amylase solution in 20 mM phosphate buffer (pH 6.9) and incubated at 25°C for 15 min. After that, about 0.5 mL of starch solution (0.8%) was added and again incubated for 15 min. The enzyme activity was stopped by adding 1.5 mL of 3,5-dinitrosalicylic acid (DNS) reagent. The solution was then made up to 10 mL using double distilled water and optical density was measured at 540 nm. Percentage inhibition was estimated by comparing the values with a control.

2.4.2. α -Glucosidase inhibition assay

About 0.1 mL of the α -glucosidase enzyme solution (25 $\mu\text{g}/\text{mL}$) was mixed with different concentrations of essential oil and incubated for 10 min at room temperature. Further, 0.05 mL of p-nitrophenyl- α -D-Glucoside (10 mM) was added and again incubated at 35°C for 30 min. At the end of incubation, 0.1 mL of sodium carbonate (w/v) solution was used to stop the enzyme activity and the optical density was recorded at 405 nm in a spectrophotometer.

2.5. Efficacy of *H. indicus* root essential oil as a biopesticide

2.5.1. Fumigant toxicity

The fumigant potential of the essential oil was analyzed against the different pests according to the method described below; briefly, the *H. indicus* root essential oil was dissolved in acetone and then coated on a Whatman No.1 filter paper having a diameter of 2 mm at their different concentrations (0–100 $\mu\text{g}/\text{L}$ air). The filter papers were then dried to remove the traces of acetone and then placed inside a glass bottle. To each of such glass bottles, 20 adult pests were released and then bottle as properly closed. The pests were observed continuously for 72 h and mortality was recorded. The LC50 value was calculated by plotting the mortality against respective doses.

2.5.2. Repellent activity

The repellency of *H. indicus* essential oil was determined according to the methods described by Kłyś et al. (2020) using a set of insect breeding containers. Briefly, the essential oil at different concentrations was dissolved in acetone and then on a Whatman no. 1 filter paper disc having a diameter of 8 mm. The disc was placed in the perforated (1.5 mm) inside the container along with wheat grains (5 g) and 20 adult insects. The repellency was estimated as the number of insects moving away from the inside container through the perforation to the outside. The median repellent dose (RD 50) was determined by plotting the essential oil concentration against the repellency at each concentration.

2.5.3. Contact toxicity

The contact toxicity of essential oil was estimated according to the methods described by Patiño-Bayona et al. (2021). The different concentrations of essential oil were applied topically using a micro-syringe on the prothorax region of adult insects (10 nos/treatment group) and the treated insects were then kept in a glass bottle. A set of insects were kept as untreated control; the toxicity up to 48 h was recorded for the calculation of LD50 value.

2.6. Effect of essential oil on germination potential of grains

The phytotoxic potential of the essential oil was determined according to the methods of Liu et al. (2006). Three different concentrations of essential oil were prepared (100, 250 and 500 $\mu\text{g}/\text{mL}$) and approximately 5 mL of these was transferred to Petri dishes containing filter paper and seeds (30 seeds). The dishes were then sealed and incubated at 25 ± 1 °C for 48 h. The seal was removed and the plates were allowed to remain at room temperature for 6 days and readings were taken every 24 h. The percentage germination in each dose and negative control was estimated.

2.7. Statistical analysis

The essential oil isolation was conducted in three independent extractions and they were compared for chemical constituents. The result of biocide studies was expressed as Mean \pm SD of five independent analyses.

3. Results

3.1. Yield and chemical composition analysis by GCMS

The average yield of essential oil by steam distillation from the root of *H. indicus* was about $1.28 \pm 0.11\%$. The predominant compound that was present in the plant was 2-hydroxy-4-methoxy benzaldehyde (92.3%); apart from that, nerolidol and camphor were the other important compounds that were present in the essential oil (Table 1).

3.2. Antioxidant effects, anti-inflammatory and anti-diabetic potentials

The antioxidant potential of the essential oil was estimated as radical scavenging and ferric reducing powers (Fig. 1). The IC₅₀ values of DPPH radical scavenging and ABTS scavenging assays were 51.38 ± 0.39 and 48.94 ± 0.64 $\mu\text{g}/\text{mL}$. The hydrogen peroxide scavenging potential was found to be 64.93 ± 0.59 $\mu\text{g}/\text{mL}$ (Fig. 1a).

Table 1

The percentage composition of root essential oil of *Hemidesmus indicus* isolated by steam distillation; composition was determined by GC-MS analysis.

Sl. No	Compounds	Retention Index	Percentage Composition
1	Salicylaldehyde	1004	0.4
2	1,8-Cineol	1011	t
3	Camphor	1105	1.2
4	Pinocarveol	1110	0.1
5	β -pinene oxide	1119	t
6	Pinocavone	1126	0.1
7	Borneol	1140	0.1
8	4-Terpenenol	1152	t
9	Myrtenal	1154	0.3
10	α -Terpineol	1164	0.2
11	Myrtenol	1170	t
12	Linalyl acetate	1194	0.4
13	Isobornyl acetate	1257	0.1
14	2-Hydroxy-4-methoxybenzaldehyde	1294	92.3
15	Dihydrocarvyl Acetate	1305	0.4
16	α -Terpinyl acetate	1325	0.1
17	β -Elemene	1370	t
18	cis-Caryophyllene	1427	0.6
19	Isocaryophyllene	1456	0.3
20	β -Selinene	1457	0.1
21	Nerolidol	1560	3.1

t-indicate trace amounts only.

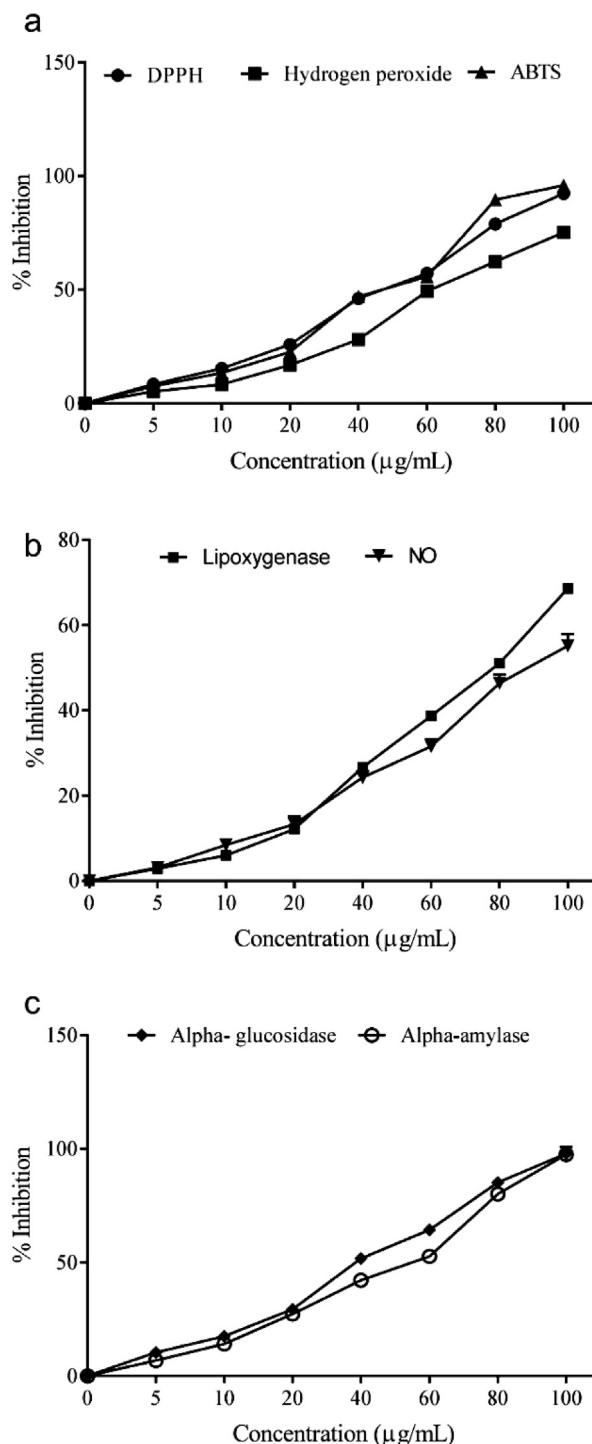


Fig. 1. Exploration of the *in vitro* antioxidant (a), anti-inflammatory (b) and antidiabetic (c) properties of the *H. indicus* essential oil. The antioxidant property was estimated as DPPH, ABTS and hydrogen peroxide scavenging; anti-inflammatory potential was represented by lipoxygenase inhibition and nitric oxide scavenging. Alpha amylase and alpha glucosidase inhibition was considered as antidiabetic activity.

Anti-inflammatory effects were estimated in terms of nitric oxide scavenging with IC₅₀ value 88.83 ± 1.21 $\mu\text{g}/\text{mL}$ and lipoxygenase inhibitory potential was 75.48 ± 0.78 $\mu\text{g}/\text{mL}$ (Fig. 1b). Alpha-glucosidase and alpha-amylase inhibition was observed with IC₅₀ values 44.71 ± 1.55 and 50.89 ± 0.86 $\mu\text{g}/\text{mL}$ (Fig. 1c).

3.3. Repellency, fumigant and contact toxicity of *H. indicus* essential oil

The pesticide property of *H. indicus* root essential oil was evaluated in terms of the repellency potential, fumigant toxicity and contact toxicity. The repellent effect was high against the *S. oryzae*, followed by *C. maculatus* and *T. castaneum* with respectively IC₅₀ values 6.08 ± 0.26 , 10.16 ± 0.63 , and 7.59 ± 0.28 $\mu\text{g/L}$ of air (Table 2). Likewise, the fumigant potential of the essential oil was high against *S. oryzae* (22.6 ± 0.87 $\mu\text{g/L}$ of air). On the contrary, the contact toxicity was high against *C. maculatus* (78.2 ± 4.58 $\mu\text{g/mg}$).

3.4. Phytotoxicity of essential oil on germinating seeds

Pesticides are not usually devoid of secondary effects on the grains and pulses. Therefore, the phytotoxic effect of the essential oil against the wheat grains was evaluated. However, there observed no significant toxicity in terms of germination of wheat grains in the root essential oil of *H. indicus* for 144 h (Table 3).

4. Discussion

The *Hemidesmus indicus* commonly known as Indian sarsaparilla is a shrub widely used in dietary beverages; the roots of the plant are also used in the traditional medicines as decoctions. Despite their wide usage and product range, limited reports were available on the essential oil of *H. indicus*. The chromatographic analysis indicated the presence of nerolidol and camphor, and to a significantly higher concentration, 2-hydroxy-4-methoxy benzaldehyde. Previous studies by Jirovetz et al. (2002) also indicated a similar pattern of GCMS and increased level of 2-hydroxy-4-methoxy benzaldehyde in *H. indicus* root essential oil.

The antioxidant potential of the essential oil was identified in terms of the multiple radical scavenging abilities. The free radicals are thought to initiate various cellular reactions that are causing damages to macromolecules like proteins, enzymes, cell membrane lipids and even nuclear materials (Alara et al., 2019). Hence, the scavenging of these radicals often protects the cells from various noxious insults. Inhibition of nitric oxide and lipoxygenase is also important in terms of the inflammatory reduction ability of the essential oil (Alzarea et al., 2021). Together, the antioxidant and anti-inflammatory properties of *H. indicus* essential oil makes it a possible pharmacological agent. Inhibition of carbohydrate metabolic enzymes α -amylase and α -glucosidase contribute to its anti-diabetic properties; therefore, intake of *H. indicus* essential oil may also help to regulate the body glucose levels (Patel and Ghane, 2021).

Table 2

The repellent efficacy, contact toxicity, and fumigant toxicity of *Hemidesmus indicus* root essential oil isolated by steam distillation against different pests of stored products. The values represented are the half-maximal inhibition concentration of each activity.

Test	Assay	Steam distillation
Fumigant toxicity LC ₅₀ ($\mu\text{g/L}$ of air)	<i>Sitophilus oryzae</i>	22.6 ± 0.87
	<i>Tribolium castaneum</i>	29.5 ± 0.74
	<i>Callosobruchus maculatus</i>	24.4 ± 0.15
Repellent activity RC ₅₀ ($\mu\text{g/L}$ of air)	<i>Sitophilus oryzae</i>	6.08 ± 0.26
	<i>Tribolium castaneum</i>	10.16 ± 0.63
	<i>Callosobruchus maculatus</i>	7.59 ± 0.28
Contact toxicity LD ₅₀ ($\mu\text{g/mm}^2$)	<i>Sitophilus oryzae</i>	93.6 ± 2.07
	<i>Tribolium castaneum</i>	88.1 ± 4.16
	<i>Callosobruchus maculatus</i>	78.2 ± 4.58

Among the different groups of insects, numerous species are known to be deleterious to humans in terms of health and economy. Vectors of diseases are generally considered deleterious to humans and plants; however, the pests, which are known to cause serious damages to the crops and products are also causing significant decline with respect to the productivity and thereby the economic status of a country. The pests of stored products are a major threat as they are usually causing damages to the preserved materials. The results of the study indicated the possible use of essential oil from *H. indicus* as a repellent against the *S. oryzae*, *C. maculatus*, and *T. castaneum*, the common pests of stored products. Further, the essential oil has also been found to be efficient to induce mortality in pests as a fumigant and contact insecticide. Previous studies have also indicated the possible role of essential oil derived volatile compounds as possible pesticide candidates against various pests of stored grains (Singh et al., 2021). It is also reported that benzaldehyde and its derivatives (the predominant compound of the essential oil is 2-hydroxy-4-methoxy benzaldehyde) are known for their efficacy as antimicrobial and insecticidal compounds (Ullah et al., 2015). It is therefore possible that the bioactive compound of *H. indicus* essential oil is responsible for its biocidal properties; further purification of the compound may yield a novel insecticidal compound.

Pesticides are often known to cause significant environmental toxicants and are also deleterious to human health. Previous studies have indicated that fumigant pesticides like phosphine and methyl bromide often induce neurotoxicity and also induced leukaemia in humans (Alavanja et al., 2014; Park et al., 2020; Van de Sijpe et al., 2020). It was also known to cause significant phytotoxicity mediated through genotoxic effects on plants (Fatma et al., 2018). However, the root essential oil of *H. indicus* is found to be safer and no sign of toxicity was observed in the germinating grains was observed during the study.

The study thus concludes that the root essential oil of *H. indicus* is a novel repellent and insecticidal compound against the pests of stored products. They can be utilized to minimize the effect of pest-induced damages to the crops and economy to an extent. Further, the non-toxic nature of the essential oil makes it an eco-friendly option to control these pests.

5. Conclusion

The *Hemidesmus indicus* root essential oil was found to be a strong antioxidant agent by inhibiting various types of free radicals. In addition, the anti-inflammatory potential and anti-diabetic effects of the essential oil increases its pharmacological values. Further, it is an effective biocide against the different pests of stored food materials. The fumigant potential oil of the essential oil enhances its application even in food storage houses. Apart from that, there observed no significant variation between the germination potential of grains exposed to the different doses of the essential oil. It is thus possible that *H. indicus* root essential oil may be an effective and eco-friendly biocide against different pests of stored grains.

Declaration of Competing Interest

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

Acknowledgement

The authors acknowledge King Saud University, Riyadh, Saudi Arabia for funding this research through Researchers Supporting

Table 3The phytotoxicity of *Hemidesmus indicus* root essential oil against the wheat grain germination index (percentage of germination) from 48 h till 144 h.

Duration of exposure in hours	Negative control	<i>Hemidesmus indicus</i> essential oil ($\mu\text{g/mL}$)		
		100	250	500
48	16.4 \pm 2.4	12.9 \pm 2.2	14.5 \pm 1.7	14.1 \pm 1.6
72	36.1 \pm 1.8	34.5 \pm 2.1	35.2 \pm 1.1	32.7 \pm 1.6
96	68.7 \pm 1.9	61.0 \pm 2.0	69.6 \pm 2.2	65.2 \pm 2.0
120	85.4 \pm 1.2	88.3 \pm 2.6	84.6 \pm 2.4	85.2 \pm 1.3
144	90.1 \pm 2.7	88.6 \pm 2.4	90.4 \pm 1.3	91.0 \pm 1.5

Project No: RSP 2021/11. The authors are also thankful to DBT-STAR scheme and RUSA grants for the infrastructural development support.

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