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PHARMACOLOGICAL ACTIVITIES OF MEDICINAL PLANTS

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ABSTRACT

In pharmacology, biological activity or pharmacological activity describes the beneficial or adverse effects of a drug on living matter. When a drug is a complex chemical mixture, this activity is exerted by the substance's active ingredient or pharmacophore but can be modified by the other constituents. Among the various properties of chemical compounds, pharmacological/biological activity plays a crucial role since it suggests uses of the compounds in the medical applications.

KEYWORDS: *Hyptis suaveolens* and pharmacological activity.

INTRODUCTION

Herbal preparations are used in traditional medicine as crude drugs in various dosage forms, as whole, crushed, powdered forms, decoctions, dried extracts, infusions, poultices and tinctures. Many of these plants have been investigated in recent times and found to contain active substances that are medically useful, whereas many more are yet to be scientifically investigated. A number of plant extracts used by natives in various parts of the world as arrow and ordeal poisons were later found to contain cardiac glycosides useful for the treatment of heart failure while others like physostigmine from the seed of Physostigma venenosun from Nigeria affect cardiac functions and are used in ordeal trials. The **thyroid gland** or simply, the **thyroid** is one of the largest endocrine glands. The thyroid gland is found in the neck, below the thyroid cartilage (which forms the laryngeal prominence, or "Adam's apple"). The thyroid gland controls how quickly the body uses energy, makes proteins, and controls how sensitive the body is to other hormones. It participates in these processes by producing thyroid hormones, the principal ones being triiodothyronine (T_3) and

thyroxine which can sometimes be referred to as tetraiodothyronine (T_4). These hormones regulate the growth and rate of function of many other systems in the body. T_3 and T_4 are synthesized from iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homeostasis.

Arthritis (from Greek arthro-, joint + -itis, inflammation; plural: arthritides) is a form of joint disorder that involves inflammation of one or more joints. There are over 100 different forms of arthritis. The most common form, osteoarthritis (degenerative joint disease), is a result of trauma to the joint, infection of the joint, or age. Other arthritis forms are rheumatoid arthritis, psoriatic arthritis, and related autoimmune diseases. Septic arthritis is caused by joint infection.

The cholesterol lowering was frequently used as a surrogate endpoint for beneficial effects on CVD, counter-examples exist where specific medications for cholesterol lowering were found to have unfavorable effect on clinical endpoints, such as with ezetimibe. Phytosterols further reduce cholesterol levels by about 9% to 17% in statin users. The type or dose of statin does not appear to affect phytosterols' cholesterol-lowering efficacy. Because of their cholesterol reducing properties, some manufacturers are using sterols or stanols as a food additive.

Glycation or the Maillard reaction is the no n-enzymatic adduct formation between amino groups (predominantly the ε-amino group of lysine and the guanidine group of arginine) and carbonyl groups of reducing sugars or other carbonyl compounds. Glycation has been confirmed to have a significant role in the development of chronic diabetic complications including cataract (Singh *et al.*, 2001) and normal aging. Non-enzymatic glycation of proteins by reduction with carbohydrates and further rearrangements, eliminations and oxidations would result in the formation of advanced glycation end products (AGEs), which might alter peptide structures, functions and stability. There have been many reports on the accumulation of AGEs under several pathophysiological conditions such as diabetes and aging.Therefore, searching a potential anti-glycated agent has drawn much attention from researchers. Till now, publications on the anti-glycated activity of polysaccharides derived from natural sources are very limited.

Biofi lms are organized into structured communities embedded within a matrix of extracellular material that is produced by the biofi lm cells (DONLAN, 2002). Generally,

biofi lm matrix composition contains water, carbohydrates, proteins, phosphorus, glucose and hexosamines (BAILLIE and DOUGLAS, 2000;). Biofi lms facilitate the adherence to the tissue and colonization of the Candida species and protect the microorganism from host defenses. Also, the formation of Candida biofi lms has important clinical repercussions because of their increased resistance to antifungal therapy (RAMAGE et al., 2005). Therefore, biofi lm formation is believed to play an important role in the pathogenesis of candidal infections in humans (YANG, 2003).

Plant name: *Hyptis suaveolens*

Scientific classification

Kingdom: Plantae

Order: Lamiales

Family: Lamiaceae

Genus:*Hyptis*

Species: ***H. suaveolens***

Binomial name: ***Hyptis suaveolens*** (L.) Poit. 1806

Erect, strongly aromatic branched annual or short-lived perennial herb up to 3 m tall. Stems 4-angled, velvety with longer hairs and gland dots. Leaves opposite, ovate, 2.5-10 cm long, cordate at the base, velvety on both sides, margin toothed; petiole 1-7 cm long. Young leaves often purple tinged, particularly on the margin. Flowers axillary pedunculate clusters. Calyx with 5 spine-like teeth, often dark purple, glandular. Corolla 2-lipped, mauve with dark purple lines at the base of the broad 2-lobed upper lip.

Chemical Contents

Plant contain many bio-active chemicals, mainly 1, 8-cineole (32%) and b-caryophyllene (29%), a-Thujene, a-Pinene, Camphene, Sabinene, b-Pinene, Myrcene, a-

Phellandrene, 1, 8-Cineole, g-Terpinene, a-Terpinolene, Linalool, Fenchol, 4-Terpinenol, a-Terpineol, Eugenol, a-Copaene, b-Elemene, b-Caryophyllene, a-Humulene, a-Bergamotene, Aromadendrene, g-cadinene and d-cadinene.

Properties

The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, sudorific, galactogogue and as a cure for parasitic cutaneous diseases(The Wealth of India 1964) . Crude leaf extract is also used as a relief to colic and stomachache. Leaves and twigs are considered to be antispasmodic and used in antirheumatic and antisuporific baths(Kirtikar et al., 1991) , an antiinflammatory, antifertility agents(Mahesh, S., 2001), and also applied as an antiseptic in burns, wounds, and various skin complaints. The decoction of the roots is highly valued as appetizer and is reported to contain urosolic acid, a natural HIV-integrase inhibitor(Chatterjee, A. and Pakrashi, S.C.,1997) . Fumes of the dried leaves are also used to repel mosquitoes and control insect pests of stored grains. In the present work an attempt was made to study the antimicrobial activity of different leaf extracts of *Hyptis suaveolens* (L.) Poit.

Plant pacifies vitiated tridoshas, cough, asthma, bronchitis, fever, toxins, vomiting, lumbago, gastric distension, urinary retention, ringworm, viral and bacterial infections and skin diseases.

MATERIALS AND METHODS

Assessment of *In Vitro* Anti-Arthritic Activity

Inhibition of protein denaturation method

The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of isolated compound (100 and 250 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. The samples were incubated at 37°C for 20 min and then heated at 57°C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

Percent inhibition= 100- (O.D. of test – O.D. of product control) X 100

IN-VITRO ANTI THYROID ACTIVITY

Assay of thyroid Peroxidase (TPO) activity: A 10 percent homogenate was prepared using human thyroid tissues collected from the ENT Department, S.S.K.M. Hospital, Kolkata; in phosphate buffer (*p*H 7.2, 100m*M*) and sucrose solution (500 m*M*) at 4ºC. Homogenization was carried out in a glass homogenizer. The homogenate was centrifuged at 1000rpm for 10 min and this low speed supernatant was further centrifuged at 10,000 *g* for 10 min at 4ºC to get the mitochondrial fraction. The microsomal fraction containing most of the peroxidase activity was obtained by centrifuging the post mitochondrial supernatant at 1,05,000 *g* for one h. Immediately after centrifugation the precipitate was solubilized in phosphate buffer. Thyroid peroxidase activity was measured using Spectrophotometer at 353nm by the method of Alexander. The tissue protein level was determined by the method of Lowry *et al* using bovine serum albumin as standard. The results are expressed as change in optical density (*ƒ*OD)/min/mg protein.

Assay of anti-TPO activity of plant: Edible part of each fresh plant (raw, boiled and cooked) was homogenized in assay buffer (5 mg plant tissue in 5 ml phosphate buffer) and centrifuged at 700 *g* for 10 min. After centrifugation fixed amounts of aliquot of the supernatant of raw, boiled and cooked plant were added separately in a 1ml cuvette containing acetate buffer, potassium iodide, microsomal fraction of thyroid tissue and hydrogen peroxide was added to start the reaction. The TPO activity (*ƒ*OD/min/mg protein) was measured following the procedure of Gaitan *et al*. Anti-TPO activities of the plant extracts were also studied in presence of excess potassium iodide. For this purpose in the cuvette maintaining the same concentration of assay buffer, plant extract (raw, boiled and cooked) and H2O2, the concentration of potassium iodide was increased (until highest activity obtained) and change in optical density (*ƒ*OD)/min/mg protein was recorded.

Invitro Anti Lipidemic activity

The sample was treated with blood serum from cholesterol patient and it was used for the analysis of Cholesterol (**Zak's Method**).

IN-VITRO ANTIGLYCATION ASSAY

Antiglycation assay was performed according to the methods reported by Matsuura and colleagues with slight modification (Matsuura et al., 2002). In all experiments, the final reaction volume was 1 ml and was performed in 1.5-ml Eppendorf tube. Albumin (1 mg/ml final concentration) was incubated with glucose (500 mM final concentration) in the presence

of sample or PBS as control buffer at specified concentration. The reaction was allowed to proceed at 60oC for 24 hours, and thereafter, stopped by adding 10 µL of 100% (w/v) Tricholoroacetic acid (TCA). The TCA-added mixture was kept at 4oC for 10 minutes before subject to centrifugation at 10000 g. The precipitate was redissolved with alkaline PBS (pH 10) and immediately quantitated for the relative amount of glycated BSA based on fluorescence intensity by spectrofluorometer F-4500 (Hitachi, Japan). The excitation and emission wavelengths used were at 370 nm, and 440 nm, respectively. In time-course studies, the reaction was allowed to proceed to different time-points as indicated before the reaction was stopped by TCA and relative fluorescent intensity was measured.

$$\% \text{ Activity} = (\text{OD Control} - \text{OD Treated}) / \text{OD Control} \times 100$$

BIOFILM INHIBITION ASSAY

The sample was dissolved in deionised water at different concentrations (20, 50 and 100 µg /ml) in sterile screw cap vials, shaken well to obtain complete homogenous mixture and were used for biofilm inhibition assay. Biofilm inhibition was carried out in modified spectrophotometric assay. 100µl of *E coli* cell suspension was prepared and added into tubes and different concentrations of sample (20, 50and 100 µg/ml) was added and the plates were at incubated 37º C for 3 days. After the incubation, the liquid suspension was removed and 100 µl of 1% w/v aqueous solution of crystal violet was added. Following staining at room temperature for 30 minutes, the dye was removed and the tubes were washed thoroughly and 95% ethanol was added and incubated for 15 minutes. The reaction mixture was read spectrophotometrically at 570 nm. Inhibition of biofilm formation was calculated by using the following formula.

$$\% \text{ inhibition} = (\text{OD Control} - \text{OD Treated}) / \text{OD Control} \times 100$$

RESULT AND DISCUSSION

In this present investigation the **Anti arthritic Activity, Antilipidemic activity, Anti Thyroidal Activity, Antiglycation Activity and Bioflim inhibition assay** of ethanolic extract of *Hyptis suvelons* was examined by *Invitro method.*

***Invitro* Anti arthritic Activity**

Anti-arthritic effect of sample studied significantly by using *in-vitro* inhibition of protein denaturation model. The effect of *Hyptis suvelons* extract inhibition of protein denaturation

was shown in Table-2. Extract of *Hyptis suvelons* extract at two different concentrations (dose levels) provided significant protection against denaturation of proteins. The plant extract possess 9.1% whereas the control contains 3.7% of Protection was observed respectively.

Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Obtained data stated that selected sample could be used as potent anti-arthritic agent.

***Invitro* Antilipidemic activity**

In this present investigation the *Hyptis suvelons* leaf extract may have acted through one of the above mechanisms. Serum lipids are known to be elevated during severe cholesterol patients and have been implicated in the following techniques. The regression of the cholesterol state due to the administration of the *Hyptis suvelons* leaf extract may have decreased the level of cholesterol when compare to control, thereby depressing the mobilization of fat. The cholesterol content of plant extract used blood possesses 55.9mg of Cholesterol whereas the control blood possesses 63.2mg of cholesterol was observed respectively.

***Invitro* Anti Thyroidal Activity**

The activity of the enzyme was reduced in presence of the *Hyptis suvelons* possess (16.6%) whereas the control contains 40% respectively. In presence of extra iodide recovery in TPO activity was maximum with *Hyptis suvelons* when compare to control. The results are described in (Table 2).

Many vegetables and plants containing cyanogenic constituents are often consumed but the information on the systemic quantification of different goitrogenic/anti-thyroid components of these vegetables of Indian origin is scanty. Marked variations were noted in the observations apparently for differencess in genetic backgrounds and ecological factors and also for presentations of data. Cyanogenic glucosides, glucosinolates and thiocyanate are known as goitrogenic principles of cyanogenic plants. Goitrogenic/ antithyroidal potential of a plant food depends not only on the nature and the relative concentration for these

goitrogenic principles present in it but also on how it is processed as food or the iodine nutritional status of the body.

Raw extract of selected sample reduced TPO activity from 16.6%. Thiocyanate or thiocyanate like compounds primarily inhibit iodide concentrating mechanism of the thyroid, at high concentration thiocyanate inhibits the incorporation of iodide into thyroglobulin by competing with iodide at the thyroid peroxidase level and forming insoluble iodinated thyroglobulin in thyroid24. High concentration of thyocyanate is also responsible for inhibition of TPO catalysed oxidation (I- leads to I2)6 while glucosinolates undergo a rearrangement to form isothiocyanate derivatives4. Isothiocyanate reacts spontaneously with amino groups to form thiourea that interferes in thyroid gland with organification of iodide and formation of thyroid hormone and this action cannot be antagonized by the iodide. Thus, the in vitro inhibition of TPO activity of raw extract seen in the present study appeared to be mediated through thiocyanate or isothiocyanate like anti-thyroid derivatives. In the present study the plants reduced the TPO activity.

***In vitro* Antiglycation Activity**

The concentrations of extracts tested for antiglycation activities range in 50µg/mL (Table 3). Results showed that the selected sample possessed antiglycation activities compared to the control experiment (without the extracts added) and comparable to the anti-glycation drug aminoguanidine. From the results obtained, it was observed that the degree of antiglycation activities varies considerably from plant to plant and also on the different fractions tested. The *Hyptis suvelons* showed the highest percentage activity (98.63) at 50µg/mL.

Invitro biofilm activity

Biofilm production in NA broth was much greater than that observed in Hyptis suvelons. In NA most of the serotypes were found to be strong and moderate biofilm producers, while in Hyptis suvelons they produced weak biofilm. The same patterns of differences were also observed among other serotypes of E.Coli in both the growth medium (Figures 1 and 2). Therefore, NA broth was selected as the medium for biofilm formation in this study.

Incubation period

At 48 h of contact time none of the E.coli strains formed biofilms Concentration of sample 20µg, 50µg and 100µg and the average OD ranged from 0.43, 0.34 and 0.10 which was equal

to the control OD (i.e. 0.43 ±.03). The results were observed after 48 h of incubation and were considered sufficient to evaluate the ability of E.coli serotypes to produce biofilm.

Table 1: *In Vitro* Anti-arthritic activity of Hyptis suvelons

Samples	Inhibition values in %
Control	**3.7**
Hyptis suvelons	**9.1**

Table 2: *In Vitro* Anti-lipidemic activity of Hyptis suvelons

Samples	Cholesterol values in mg
Control	63.2
Hyptis suvelons	55.9

Table 3: Biofilm Inhibition Assay

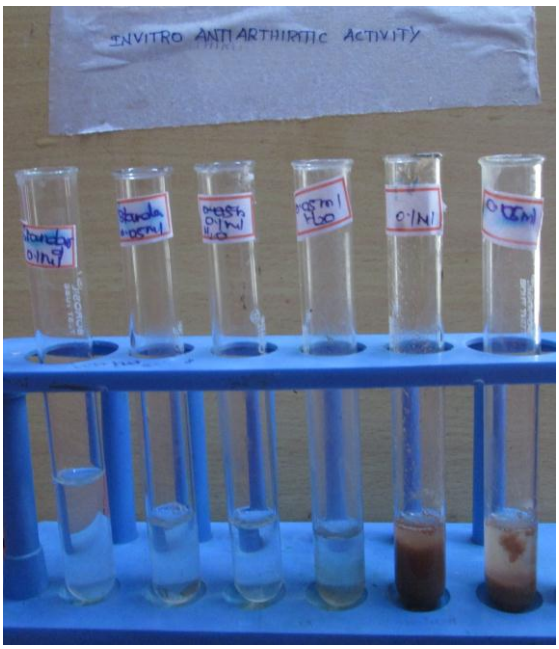
Microorganism	% of Inhibition (*Hyptis suvelons*)		
	20µg /ml	50µg /ml	100µg /ml
E. coli	13.6	30.8	58.8

Table 4:- Estimation of Invitro Antithyroid activity

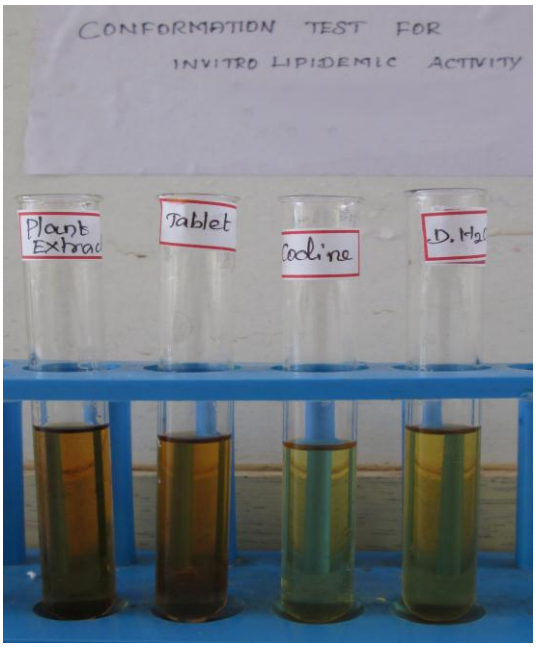
S. No	Samples	Values in %
1	**Control**	40
2	Hyptis suvelons	16.6

Table 5: Anti Glycation Activity of *Hyptis suvelons*

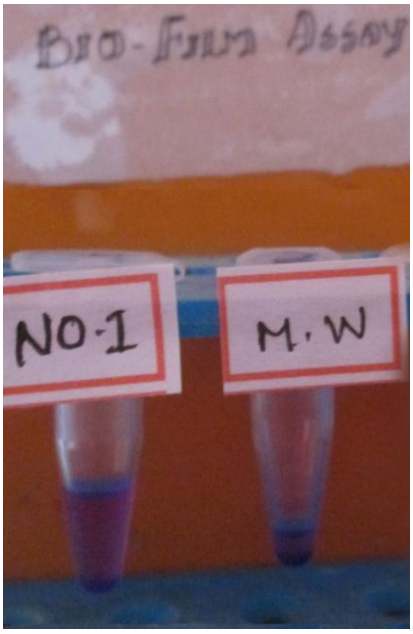
S. No	Samples	% of Activity
1	*Hyptis suvelons*	98.63



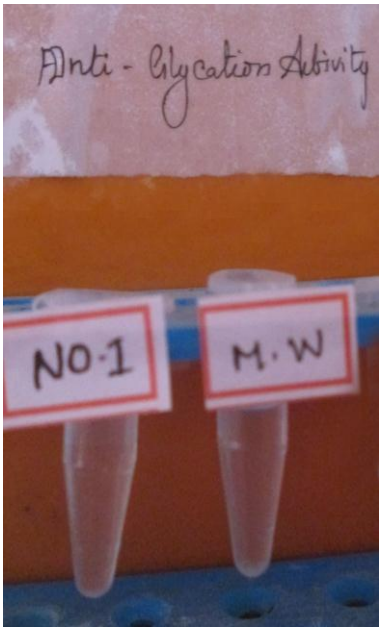
Antiarthiritic activity



Anti Lipidemic activity



Bio flim assay



Anti glycation activity

Anti thyroidal activity

The results of our study are in agreement with other studies, where 48 h of incubation period was suggested for biofilm formation in E.coli .(Brendan and Ethan, 2005; Joseph et al., 2001). At 72 h PI decrease in OD was observed, which indicated that the saturation level was already reached. Some researchers also used prolonged incubation of 96 h to 10 days for biofilm formation (Hood and Zottola, 1997; Prakash and Krishnappa, 2002; Malcovaa et al., 2008).

Glycation and AGE-induced toxicity are known to be associated with increased free radical production. Therefore, agents that possess good antioxidant activity by mopping up free radicals can simultaneously inhibit the formation of advanced glycation endproducts (Ahmad *et al.*, 2006).

Hence the present study confirms the significant antihyperlipidemic potential of three plants of *Hyptis suvelons* owing to its ability to reduce level of total cholesterol, triglycerides, LDL and increasing HDL level. *A. viridis* showed highly significant anti-hyperlipidemic activity compared to *A. caudatus* and A. spinosus in triton induced hyperlipidemia in rats. Further studies to isolate, identify and characterize the active principle(s) are in the progress. The antihyperlipidemic activity may be attributed to some of its active principles.

Raw extract of all the plants reduced TPO activity from 60 to 85 per cent. Cyanogenic glucosides are readily converted into active goitrogenic agent thiocyanate by glucosidases

and sulphur transferase enzymes present in the plant or in the animal tissues. Thiocyanate or thiocyanate like compounds primarily inhibit iodideconcentrating mechanism of the thyroid, at high concentration thiocyanate inhibits the incorporation of iodide into thyroglobulin by competing with iodide at the thyroid peroxidase level and forming insoluble iodinated thyroglobulin in thyroid (Van Middlesworth L., 1985). Thus, the *in vitro* inhibition of TPO activity of raw extract seen in the present study appeared to be mediated through thiocyanate or isothiocyanate like anti-thyroid derivatives.

SUMMARY AND CONCLUSION

Medicinal plants are of great importance to the health of individuals and communities. Medicinal plants have curative properties due to the presence of various complexes chemical substances of different composition. Phytochemicals are secondary metabolites in one or more parts of the medicinal plants. These have the ability to produce a definite physiological action on the human body. In conclusion, *Hyptis suvelons* extracts possess potent antioxidant, antiglycation properties which tend to support their use in the traditional medicines. The mechanism of action by which the extracts inhibited glycation is undoubtedly complex. However, it can be argued that the anti-glycation capacity was linked to the antioxidant potentials. Hence, it can be suggested that these plants might have possible prophylactic and therapeutic potentials in the management of diabetes and related complications. Certainly, more studies related to the structure of these phytochemicals and the mechanism(s) of action are required in order to understand their anti-glycation and antioxidant effects. In the present study, we detected a relationship between the severity of clinical signs and biofi lm activity for a few biofilm positive E.coli isolates. However, this finding was not sufficient to make a comment. Therefore, our study may be improved for the detection of the relationship between the biofilm activity of different Concentration using E.coli species and clinical signs in mastitis. We believe that this study may be a helpful tool for further research into mastitis caused by E.coli species. Arthritis is a form of joint disorder that involves inflammation of one or more joints. The most common form, osteoarthritis (degenerative joint disease), is a result of trauma to the joint, infection of the joint, or age. Other arthritis forms are rheumatoid arthritis, psoriatic arthritis, and related autoimmune diseases. Septic arthritis is caused by joint infection. The present study was concluded that the plant extracts has got profound steroids and may have potential use in medicine. Further studies are needed to isolate the exact active component, which are responsible for the medicinal agents. Thus, the study as certain the value of plants used in Ayurveda, which could be of considerable interest to the

development of new drugs. In conclusion, the results of this study show that ethanolic leaf extract/fractions of *Hyptis suvelons* possessed antiarthritic and antilipidemic properties. This confirmation justifies its use in ethno medical medicine for the treatment of arthritis and cholesterol patients.

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