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Evaluation the Active Substances of Extracts of *Syzygium Aromaticum* Plant Flower Buds on *Enterobius Vermicularis*: In Vitro Study

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Annotation: The flower bud of the Syzygium aromaticum plant exemplifies has the medical significance and it is comprise a number of active and lively compound .Present investigation was done to demonstrate the impact of cold and hot aqueous extracts of S. aromaticum (commonly named as Cloves) flower buds in paralysis and death of Enterobius vermicularis in vitro and detect utmost effective secondary compound (alkaloid, terpenoids and phenolics) of Cloves for analysis by GC-MS. Current findings appear that hot aqueous extract have a high influential where its arithmetical rate for paralysis and death of E. vermicularis about 373.08min for paralysis and 465.50min for death, with clear significance differences.

Regarding the outcome of secondary compounds of S. aromaticum flower buds, the current findings revealed that raised impacts for terpenoid compounds extract on worms in the concentration (150 µg/ml), its cause paralysis and death of worms at 7.00 along with12.00 min, respectively, followed by alkaloid compounds that cause paralysis

at 11.00 min and death of worms at 15.00min. Regarding to phenolics compounds, its cause the paralysis at 28.00 min and death of worms at 45.00 min with significance differences. The drug Ivermectin causes paralysis at 48.00 min and death at 67.0 min pinworms in the concentration (150 μg/ml), with a high impact and with significance differences. In context of GC/MS technique, its demonstrated that S. aromaticum flower buds contain eugenol , eugenyl acetate, -caryophyllene, and –humulene.

Keywords: Enterobius vermicularis, Syzygium aromaticum, secondary compound.

1-Introduction:

Enterobius vermicularis (Nematoda, Oxyuridae) parasitic helminth has common names as seat worms, pinworms, and threadworms [1,2] , it is a world helminth that influences more than 200 million subjects in the world [1] . School-age children, as well as residents in tropical regions, are among the groups most infected with this type of worm [3,1] . Enterobiosis infections are contracted by ingesting eggs containing fully complete larvae. In general, this infection is transmitted directly through the anus to the mouth through hands, water, or food that is from unsanitary sources and contaminated with eggs [2,4] , Also, these eggs may be transmitted through contaminated air and inhaled due to their minute size [5] . In fact, the increased prevalence rate of Enterobiasis could be return to several causes such as badly off personal hygiene, consumption of food or water that contaminated with fully developed eggs, evil environmental sanitation, as well as living with infected patients. [6,7] .

Parasite life cycle started by taken ova transmited from the peri-anal skin with contaminated fingers or materials, after the hatching of larvae in gut, they get maturity in the course of migration to specified area in the cecal, appendix together with adjacent parts of ascending colon [4] , after fertilization in its habitat, female emigrate to the peri-anal area to put its eggs, which give onto appears symptoms like scratching the anus region, and leading to finger contamination, perianal pruritus ,sleeping loss ,abdominal pain ,anemia, loss of appetite and teeth grinding especially during night time [4,8] .

The best diagnosis method is scotch tape (cellophane tape); mebendazole is very affective drug employed to treat of enterobiasis, pyrantel pamoate, or albendazole ,these drug are used in single dose and return after two weeks [9], also these drugs may be accompanied by some side effects like headache, metallic taste, dizziness ,nausea; morevere dud in these drugs are repeatedly reported [6].

Ivermectin (IVM) drug is one of a group of known as avermectins, a group of 16- membered, which are compounds of macrocyclic lactone and its presence has been discovered in Japan from the culture of actinomycetes with Streptomyces avermitilis [10,11]. The advantages of these drugs are that they are quickly absorbed orally, have high solubility in fats, and have a high speed of spread

in the body, vastly distributed throgh the body, easily metabolite by the liver, in addition to their easily excretion by mean of feces [12,13]. In healthy humans, after a standard oral dose, it thrusts out to peak plasma levels at about 3.4 to 5 hours, through its binds strongly to plasma proteins; thier half-life about 12–66 hours [10,13]. In fact, the Ivermectin considered a secure medication and have vital impacts on large number of internal as well as ecto-parasites, it is widely second-hand in veterinary as well as human medicine [14,13]. In human, it was utilizing to handle onchocerciasis. Ivermectin is effectual in eliminating different worms diseases (ex: ascariasis, strongyloidiasis, trichuriasis, in addition to enterobiasis), in addition to its ability to eliminate parasitic skin diseases such as scabies [12 ,15 and 16].

Chemo-therapeutic factors have been applied commonly as an auxiliary, but emergence of failure as well as the development conventional antibiotics resistance had guided to screen of a number of medicinal plants for their prospective antimicrobial efficacy and host modulating impacts [1718] .

The biological activities of natural compounds included in medicinal plant are related to their constituents, such as phenolic ,polyphenol, alkaloids, terpenoids as well vital oils, lectins, ect [19] , that widly used as a natural sources for treatment and management a different illness and a principal source for a novel drug, as a results to their diversity of biomedical activities as well chemicals elements [18].

Syzygium aromaticum plant well known as "clove", is a tree with median size from the mirtaceae family, it is frequently cultivated in coastal areas and its flower buds is the most important part for medical usage [20] , clove represents a crucial medicinal plant as a results to their wide range of pharmacological impacts as antioxidant efficacy, antinociceptive, antimicrobial and antiviral activity [20,21] , it's extensive spring of compounds like flavonoids, hidroxibenzoic acid, hidroxicinamic acid and hidroxiphenyl propene; eugenol is the fundamental bio-active part of clove [22,23].

Clove had a bacteriostatic, antiviral, antimycotic, antioxidant, anticarcinogenic, anesthetic and tranquilizer properties, clove oil has a specific anti- inflammatory features due to their ability to suppress the cyclo-oxygenase2 along with lipo-oxygenase enzymes[24].

Terpenoids are the hydrocarbons of plant origin , found widely in plants especially in Zingiberaceae also present in microorganisms, algae, marine organisms, sponges, fungi, and insects [25], terpenoids compounds can be found as monoterpenes, sesquiterpenes, diterpenes, and terpenes [**26**], it is known by precious pharmaceutical characteristics and medical usages [25,26].

Gas chromatography provided with mass spectrometry (GC-MS), is a key technical, which employed to split a mixture into their single elements for recognition and quantification [27,28] this aforementioned technique is aplatform that exceedingly applied to analyze volatile complex compound [28]. GC-MS technique can proffer a fast qualitative task relays upon the probity of a compound database and quantification; its accuracy could be doubled if used the isotope standards as well as ion mode with each other [27,28] . In the samples analysis, MS detector had gave a high sensitivity and better selectivity [28] .

The aims of current investigation were to extract the active materials from *S. aromaticum* plant flower bud and examine its biological efficiency toward the parasite outside the living body versus to ivermectin medication.

2-METHODOLOGY

2-1- Collection of plant materials:

Flower buds of *S. aromaticum* have been collected from the local markets at January 2023. These buds have been gristed by electric mill to obtain softly powder which kept in plastic containers and saved in fridge.

2-1-1- Prepare the cold and hot aqueous extract::

Extract of cold aqueous has been prepared throughout mixing 10g of powder of the *S. aromaticum* plant and with 200ml of distilled water with mixing by mixer until about 30 min; after this its putted in a test tube in the centrifuge for 10 min at 3000 rolls/ min, then oven at 45C has been utilized to dry the extract to keep it in refrigerator until use. The hot aqueous extract has been prepared in the similar manner except that boiling water was used instead of cold [29] .

2-1-2-Extraction of secondary Plant component

2-1-2-1- Crude alkaloids extraction:

Filter papers fixed on thimbles were employed to extract the loose powder (10g), and then about 200 ml of ethanol (%99) was adding for 24 h by soxhlets (an apparatus showed in figure-1). Newly formed product have been concentrated using a rotary evaporator device. It resolved in ethanol (5ml) accompanied by adding of Sulfuric acid 2% (about 30 ml), with subsequently rotary evaporator device to detach the ethanol. Mayer assay provides white product to clinch a presence of alkaloids. in a in separating funnel, the Hydroxide ammonium %10 has been putted escorted by putting 10ml from chloroform; the product mixture had dividing into two layers, bottom layer was selected as it has alkaloid, it have been condensed with rotary evaporator, a newly dried products was preserved in ice-box[30] .

Figer (1) Soxhlets employed for extracting alkaloids.

2-1-2-2: Crude phenolic Extraction:

[31] method has been employed to extract phenolic, about 20g of dried extract powder was putted in a flask escorted by 400ml of %2 acetic acid employing the reflex condenser in water bath (at 70 degree centigrade) for 8 h. New suspension filtrated then placed with N-propanol along with Nacl in the suppression of separation, then top-layer having phenolic materials had taking, and it was focused with evaporator rotor and a newly dried product was preserved in refrigerator.

2-1-2-3: Crude Trpenoid Extraction:

[32] has been used to prepare crude terpenoids extract, nearly 20 g of dried powder was extracting with chloroform solvent by soxholet device utilizing 200 ml of chloroform solvents at 45C º for twenty four hours, then extracted substance was concentrated using rotary evaporators, specimen with terpenoid extract has dried up in electric oven to 45-40Cº. Dried material was conserve in closed glass bottle until usage. Reagents have been applied by mean standard procedures to reveal the existence of alkaloid, phenolic along with terpenoid.

Figer (3) sexohelts apparatus for terpenoid compounds extracted

2-1-2 -4: Essential oils extraction

Hydrodistillation has been applied to extract the essential oil from flower bud of *S. aromaticum* by completely washed by distilled water. About 500g of fruits have been transmitted to Clevenger device and adding three liters of distilled water. The device had set at boiling temperature for six hours, then the distilled fruit essential oils had collecting in a dried flask. The hydrodistillation was carried out for a three times; then fruits essential oils were attend to anhydrous sodium sulphate that withdraw residual moisture; then preserved in a closed tube at 4°C until usage [33]. GC-MS technique was analyzed essential oils to indicate major bioactive substances found in *S. aromaticum* plant flower buds.

2-1-2-5: Chemically analyzes of essential oil via GC/MS

Flower buds essential oil has been analyzed employing GC/MS. The analysis of *S. aromaticum* plant flower buds essential oil was done on agilent technology (Little Falls, California, USA) 6890 series gas chromatography (GC) system [34].

2-2: Collection of worm sample.

The current study was carried out by collecting samples of adult *E. vermicalaris* from the anal region of children (at midnight) from Babylon Governorate. After collecting them, the pinworms were washed with distilled water and placed in petri dishes containing phosphate saline buffer inside an incubator at a temperature of 37 degrees Celsius until used [35].

2-2-1: Microscopic assay:

Using the light microscope, pinworms were diagnosed applying wet mount method for detection the adult worms or ova; the current study was done at microbiology Lab of laboratory and clinical department, pharmacy college, Babylon University.

2-3: The impacts of *S. aromaticum* extract :*In Vitro* analysis.

Stock solutions was prepared of both extracts of the *S. aromaticum* flower buds by melting one gram of dried extract in about 100ml distilled water; consequently, the stock has a 10mg/ml or 10000 µ/ml concentration.

Stock solutions have been utilized to prepare three concentrations 50,100 and150 µ /ml; control has been prepared of phosphate buffer saline only. Comparesion of cold and hot water extracts effectiveness with ivermectin drug acomplished using identical concentrations.

Pinworms were dividing into 4 groups of, each one with 5 worms, one of them considered as control group, the remaining groups have been exposed to a desired levels of extracts along with drug, times for completely palsy and die was documented. To confirm the time of paralysis, an external stimuli have been used. Paralysis times were calculated when the worm's motion was lost, while lethal time of worm was recorded when the worm died followed by dimming and fading of the body color [36].

2-3-1: Testing the effectiveness of extracts:

Pinworms were assembled in Petri dish containing 1 ml of phosphate buffer saline, then set in incubator, after 1 h, several levels of cold, hot as well as secondary compound for *S. aromaticum* flower buds have been added to the petri dishes containing the worm by mean of adding about 1 ml of each concentrations into the petri dishes with worms. In another dishes containing worms a same concentrations of ivermectin drug has been added [35].

2-3-3: The impacts of ivermectin drug: *In Vitro analysis*

From ivermectin drug, a stock solution has been prepared through adding 6mg of ivermectin drug along with 10ml of distilled water to form 0.6 mg/ml or 600 µ/ml, and a concentrations 50,100 as well as 150 µ /ml had prepared plus to controls group, paralysis and lethal time were documented with three replicates, after this 1ml of every concentrations were added to a petri dishes which containing worms (each on its own) for the drug.

2-3-4: Assessment of worm Viability: *In Vitro analysis*

By looking, motion or death were observed; no motion or death of pinworms was identified [35] .

3-Statistical analysis:

Data of present study were analysis by mean of factorial experiments with completely randomized (C.R.D) as well as using least significance differences (LSD) at pvalue ($P \leq 0.05$) employing SPSS program.

4-The results:

Figure (1) Adult females of *E.vermicularis*(10X).

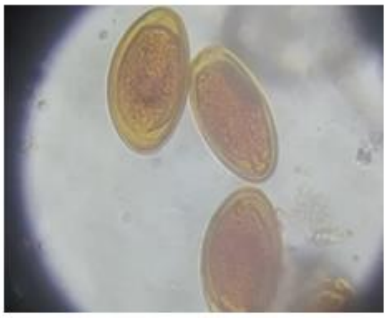


Figure (2) Ova with larvae of *E.vermicularis*(400X).

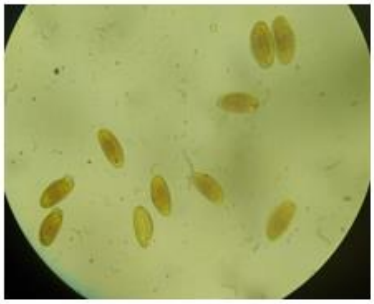


Figure (6) Eggs of *E.vermicularis* (40X).

Figure (7) adult of *E.vermicularis* (10X).

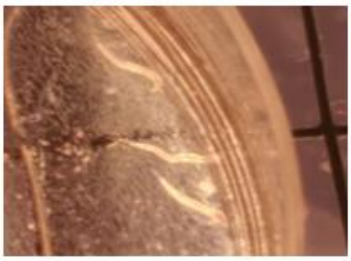


Figure (9) adult of *E.vermicularis* (20X).

Figure (10) The front portion of *E.vermicularis* body (20X).

Table (1): Impacts of *S. aromaticum* extract type (cold and boiling aqueous extracts) on worm paralysis & death *In Vitro*.

Extract	Time/min M±S.D. paralysis	Time/minute M±S.D. death
Hot extract	373.0 8 ± 168.72	465.50 ± 190.04
Cold extract	416.08 ± 156.300	519.42 ± 181.36
LSD at probality level 0.05	52.6	3.4

Table (2): Impact of overlapping concentrations of *S. aromaticum* extracts (cold and boiling aqueous extracts) on worm paralysis & death *In Vitro*.

Syzygium aromaticum plant	Extract	Concentration µ /ml	Time /min M±S.D paralysis	Time /min M±S.D death
	Hot extract	150	35.00±0.57	55.00±0.57
		100	54.00±0.57	87.00±0.57
		50	62.00±0.57	165.00±0.57
		Control	1341.33±33.09	1555.00±1.52
	Cold extract	150	75.33± 1.45	110.00±0.57
		100	104.33±1.20	160.00±0.57
		50	174.00±0.57	250.00±0.57
		Control	1310.66±36.88	1557.67±2.4
LSD at probality level 0.05			52.5	3.4

Table (3): The Interference Effect of Secondary Compound Concentrations for *S. aromaticum* plant flower bud extracts on worm paralysis & death *In Vitro*.

Secondary compounds types	Concentration µ /ml	Time /min M±S.D paralysis	Time /min M±S.D death
Terpenoids compounds	150	7.00±0.57	12.00±0.57
	100	14.00±0.57	19.00±0.57
	50	24.00±0.57	33.00±0.57
	Control	1355.33±	1556.67±2.84
Alkaloids compounds	150	11.00±0.57	15.00±0.57
	100	21.00±0.577	32.00±0.57
	50	36.00±0.57	56.00±0.57
	Control	1325.00±32.12	1555.00±2.64

Phenolics compounds	150	28.00±0.57	45.00±0.57
	100	55.00±0.57	87.00±0.57
	50	96.00±0.57	157.00±0.57
	Control	1377.00±0.57	1554.33±1.85
LSD at probality level 0.05		42.45	3.91

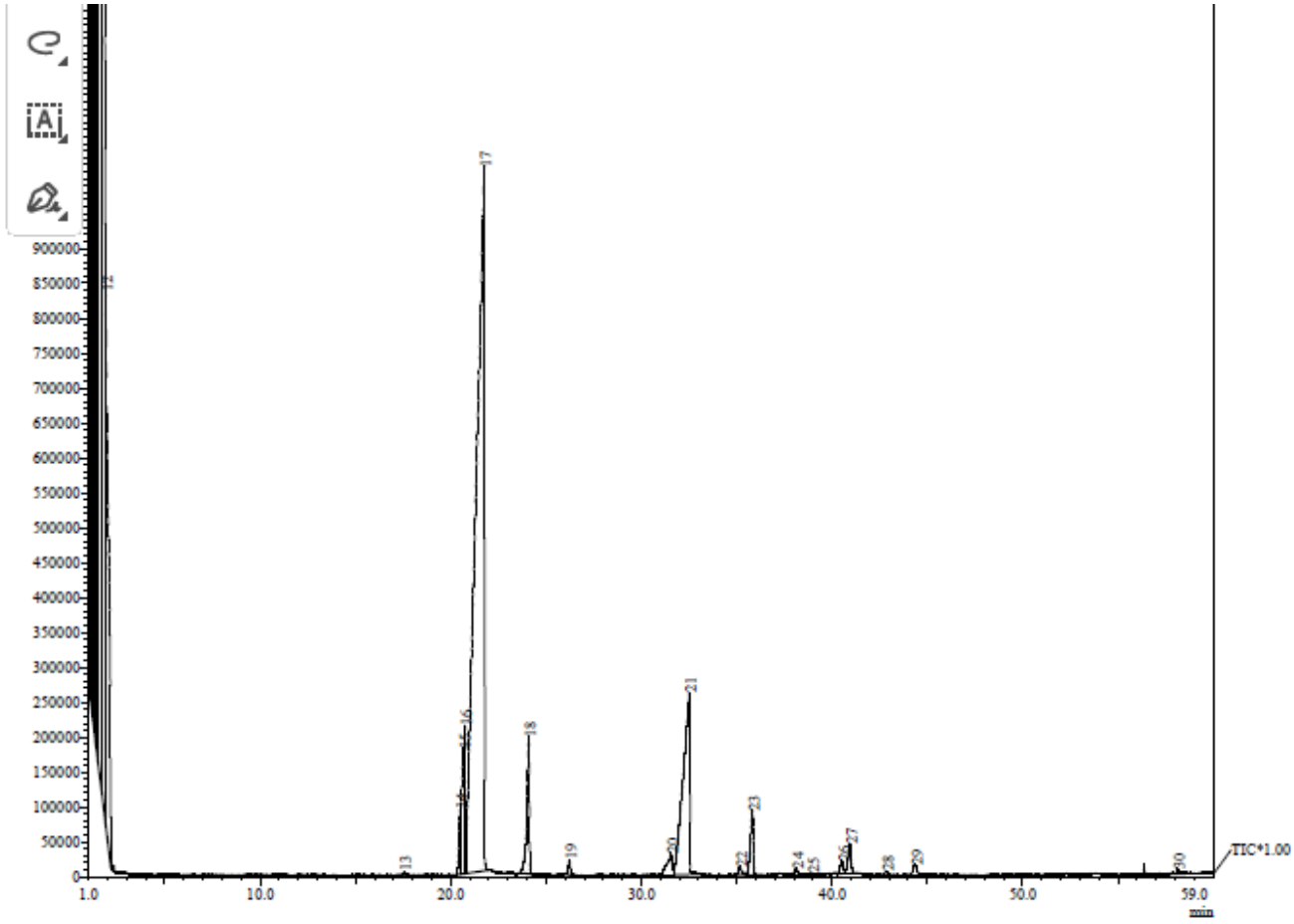
Table (4): the impact of ivermectin drug concentrations on worms paralysis & death *In Vitro*.

Ivermectin (µ /ml)	Time/min for paralysis M ± S.D	Time/min for death M ± S.D
150	48.00±0.57	67.00± 0.57
100	93.00±0.57	128.00±0.57
50	135.00±0.57	183.00±0.57
Control	1160.67±3.18	1556.33±0.88
LSD	5.43	2.2

Table (4) GC-MS analyzes of the essentials oil of *Syzygium aromaticum* plant flower buds for present investigate study.

Peak Number	**Compound**	**Retention Time / min. of *S. aromaticum* plant in present study**	**Retention Time / min. of *S.aromaticum* plant in GCMS library**
1	Hydroxylamine	1.049	1.050
2	Hydroxylamine	1.100	1.100
3	Trichloromethane	1.186	1.185
4	Hydroxylamine	1.210	1.210
5	Methane, oxybis dichloro	1.240	1.240
6	Hydroxylamine	1.349	1.350
7	Hydroxylamine	1.386	1.385
8	Trichloromethane	1.432	1.430
9	Hydroxylamine	1.473	1.475
10	Trichloromethane	1.561	1.560
11	Trichloromethane	1.729	1.730
12	Trichloromethane	1.901	1.900
13	Phenol, 4-(2-propenyl)-	17.561	17.560
14	Phenol, 2-methoxy-3-(2-propenyl)-	20.500	20.500
15	3-Allyl-6-methoxyphenol	20.660	20.660
16	Eugenol	20.752	20.750
17	Eugenol	21.765	21.765
18	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene	24.096	24.095
19	.alpha.-Caryophyllene	26.231	26.230
20	3-Allyl-6-methoxyphenol	31.550	31.549
21	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	32.519	32.520
22	Bicyclo[7.2.0]undec-4-ene,	35.19	35.190

	4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-		
23	Caryophyllene oxide	35.836	35.835
24	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl	38.140	38.140
25	Diepicedrene-1-oxide	38.956	38.955
26	Aromadendrene oxide-(2)	40.551	40.550
27	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	40.955	40.955
28	Isoaromadendrene epoxide	42.854	42.855
29	Isoaromadendrene epoxide	44.375	44.375
30	7-Hexadecenoic acid, methyl ester, (Z)-	58.149	58.150



Figer 1: Chromatogram of the essentials oil of *Syzygium aromaticum* plant flower buds of present study.

5-Discussion:

Indeed, *E. vermicularis*, that often known as pinworm has a global prevelence, with higher rates distribution in temperate regions [6] . Using the medicinal plants representing the most ancient pattern of treatment, had applied since thousands of years in conventional medicine of different countries and for various population [38,39] ,it's treating and preventing various diseases [38]. Oils of clove has wide prospects possibility as a remedial agent and an alternative treatment against various helminths [40].

The findings of present investigation revealed a raised efficacy concerning hot water as compared

to cold water of *S. aromaticum* flower bud extracts on both paralysis and death of *E. vermicularis* worms, the present finding revealed that hot water extracts of *S. aromaticum* flower bud very effective and have the ability to cause paralysis at (373.08) minutes and destroy or killed a worm at (465.50) minutes with significance differences as found in table (1) ,these results apply with what researchers got [41,42]. who confirmed that *S. aromaticum* plant flower buds extract have an activity against the parasites, where the researchers [41]they were study the anthelmintic effect of clove on *Pheretima posthuma* Indian adult earthworms ,water extracts were prepared in three concentrations (100%, 50% and 20%), clove aqueous extract was very active than others that was lead to die worms at about 25 min in 100% concentration and murder the worms in 43 and 67 min at 50% and 20% concentration, paralysis time was 12, 32 and 45 min, respectively, for the extract of cloves; as well as [42]. they estimate the impacts of same plant extract on egg production of gastrointestinal nematodes *Trichostrongylus spp.* , *Ostertagia spp* ., *Cooperia spp* . as well as *Haemonchus contortus* in thirty sheep infected that were ordered in 10 groups of animals, the first group taken 1.8 g ground cloves for one week, the percentage of reduction of fecal egg counts was 40.7 percent on day 12 in clove group.

This effect probably demonstrates depend on that the temperature of a hot water extract of *S. aromaticum* plant fruit bud that acts or work to dissolved the active substance as terpinoids, alkaloids and phenolics secondary compounds where the raised temperature of water guides to ameliorate extraction process [43,44 and 45] . Further, raised its concentration also elevated the mass-transfer kinetics by deactivate analytes-matrix interplay particularly hydrogen bonding and other forces [46, 43 and 45] , thereby facilitating initial absorption for the analytes by the cell of organism , as well as a higher temperature resulted in faster diffusely, boosted temperature degree in water extract had a several utility as amended active materials kinetics which led to increase it is activity [46,45 and 47] , as phenolic compounds where a higher capacity of the phenolic compounds has been observed in the extracts that are obtained at raised temperatures compared to the extracts obtained at lower temperature [44,45 and 47] .

Also hot extract effect of *S. aromaticum* fruit bud could be because it is contain a higher amounts of phenolic compounds (tannins, phenols and flavonoids), terpenes and alkaloids [48] , where the phenolic compounds dissolve in water [39] , as well as the phenolic compounds may be effects on parasites body by hydrogen atoms transfer, single electron as well sequential proton losing electrons transfer, in addition of chelation of transition metals from body of organisms [49], or the phenolic compounds reactive with proteins or peptidoglycan of parasites and alter their functions as the tannins [50] , where the tannins can significantly protect against parasites species thought their stimulatory impacts on the antibodies formation [50,51], it is lead to chelation of iron from the medium to make it not available to microorganisms [52,50]. Moreover, the tannins had exhibit the ability to lessen the synthesis of fatty acid by irreversibly blocking of β-ketoacyl-ACP reductase in organisms [53,50]. The effectiveness of clove hot water extract may be due to it containing many highly effective substances that decompose to a high degree in hot water and thus lead to an effect on the activity and subsequent death of the pinworm more than what the cold water extract would do [54], extraction of bioactive compounds as secondary compounds through using a hot water extraction and minuscule extraction times gives a benefits that point out over extraction methods in terms of being faster and efficiency [47] , Secondary metabolites like terpene, phenolic, alkaloids, nitrogen and sulfur have compounds that mediate the plant defense against several herbivoares and pathogenic microorganisms along with different environmental stresses [55] , therefore the current investigation ensure the great influence of hot water extracts of *S. aromaticum* fruit bud compared with cold water extracts.

The statically analysis showed that the most influential secondary compound is terpenoids extracts of *S. aromaticum* plant fruit bud where it is leads to paralysis at (7.00 minutes) and die at (12.00 minutes) on *E. vermicularis* helminths or worm with significance differences as found in table (2) , these results similar with what researchers got[40,56],who confirmed that *S. aromaticum* plant flower buds extract have an activity against the parasites ,where [40], they test the anthelmintic

impacts of clove oil of extracted from *S.aromaticum* flower buds against adult and muscle larva of *Trichinella spiralis* worm in vitro study, where the adult worm and muscle larvae of *T. spiralis* were incubated with clove oil at different concentrations (from 5 to 500 µg/ml), they reported that clove oil had a lethal impact for the all larvae at 100 and 500 µg/ml at 24 and 16 hours ,respectively. also their study showed that adult worms and muscle larvae of *T. spiralis* that treated with 100 µg/ml of clove oil displaying a distinct morphological changes, multiple vesicles, blebs, the normal annulation losing, and destruction of the cuticle, as well as, [56], who evaluated the anthelmintic effectivity of the eugenol toward fresh samples of *Gyrodactylus* sp. , after one hour to eugenols' exposure, the mortality of *Gyrodactylus* was about 80% in the concentration 5mg/L while about 90% in10 mg/.

These outcomes may be visibly according to that fruit bud terpenoids of S. aromaticum plant changes the contents of cell as efflux of K+, salt tolerance, cellular content extravasation as well as absorption because mechanism of terpinoids compounds action includes shifting o membrane permeability with no cell lysis [57], followed by die of cell and all the organism, also the terpinoids compounds may be leads to raise of some harmful material inside the cells or it leads to decrease some substances outside the cells, terpenoids and phenylpropanoids characterized by possessing a high biological efficacy and a wide spectrum of action [21,57], as well the impact of terpinoids could be due to it is contains a high amount or concentration of eugenol compound [58] , where the eugenol compound work to disrupt the lipid portion of the membrane which turn to changes the permeability that leading to leakage of intracellular contents [59], also other potential pathways for eugenol include the induction of apoptosis by devastation mitochondria's membrane and production of reactive oxygen species (ROSs) which bring depletion of intracellular non-protein thiols and boost in the earlier lipid layer break [60], as well as it had revealed to impede with action potential conduction [59], it was demonstrated that eugenol have a several characteristics such as anti-inflammatory, antioxidant, neuro-protective, antipyretic feature, antifungal and analgesic features [59,60].

The impact of terpenoids seem to attributed to interplay among components, that are presume to be happened as a result of emission of terpenoids [18,62] , terpenoids are effective agents and are widely used as therapeutics [18] , in addition, previous investigations revealed that terpenoid can else given (either individually or mixture) due to their antioxidant propensity [Stephane and Jules (2020]. Morevere, terpenoids as principal bioactive components of essential oils [18,63] investigation confirmed that terpenoids found in *S. aromaticum* flower buds have an extraordinary impacts than other Secondary compounds.

The results of this examination seems the impact of alkaloids secondary compounds on paralysis and death of worm where it is leads to paralysis at (11.00 minutes)and die of worm at (15.00 minutes) with significance differences, these findings similar to what the researcher has confirmed of the effective ness of this extract against the parasites as [15], they *in vitro* investigated the possibility of growth inhibition impact of *S. aromaticum* alcoholic extracts against *Babesia spp.* as well as *Theileria equi* parasites, their results appears that *S. aromaticum* methanolic extracts effect were 109.8 , 8.7 , 76.4 , 19.6 , and 60 µg/ml inhibitory effect, respectively, on these parasites, this effect of the alkaloids compounds seems to return to its capacity to influence different metabolic pathways in animals, it is worthing to note, that most alkaloids toxic mechanism may be emerge by the enzymatic shifting that could influence a wide range of physiological processes, inhibiting the DNA synthesis and repair system by interrelating with nucleic acids, or influence the nervous system; generally, different alkaloids may have an effect on multiple functions in animals (64,65).

The effective action of alkaloids may be ascribed to alkaloids capacity to disrupt the enzymes action, damage the receptors and proteins by formation hydrogen bonds with their functional groups [66], such as pergularinine and tyllophorinidine alkaloids, they impede dihydrofolate reductase enzyme that engaged in synthesis of nucleic acid [67] ,some alkaloids could bind to a protein which has a functional impact on cell division with a distinct affinity and leading to suppression of cell division [67,68] ,other alkaloid maybe proceeds acting via a detergent-like pathway which guide the

perturbation of outer-membrane of pathogens [69] Furthermore, alkaloids' activity maybe a result to their ability to inhibit microorganisms' virulence factors [70].

The alkaloids are crucial therapeutic agent for their ability to prohibit the onset of numerous degenerative diseases by scavenging ROSs or engaged with catalysts of oxidative reactions [71,72], furthermore, alkaloids impede growing and development of microorganisms as bacteria, fungi, and protozoa, due to their enormous features, so, it has become very desirable in pharmaceutical formulations and may be come out as a worthy metabolite applied to treatment several mortal diseases such as cancer [72]. also, alkaloids activity seem to be attributed to inhibit of some microorganisms virulence factors [65].

Other pharmacological properties of Alkaloids involve central nervous system stimulation, anticholinergic agents oxytocic, vaso-constrictor activity, antimalarial activity [65, 72], as well as anti-inflammatory action that include the inhibit or control of principle inflammation elements [73,74].

The static analysis about the impacts of ivermectin drug (IVM) revealed that this drug have a high activity on the paralysis along with death pinworm; its lead to a paralysis at (48.00 minute) and die (67.00 minute) in short period of time with significance differences as found in table (3), these results is consistent with previous investigations [15,12], both of them verified that the ivermectin drug has a high effect on parasites where [15] they were work about the efficacy of ivermectin drug on the growth of *Babesia* sp. including *B. bovis*, *B. bigemina*, *B. divergens*, *B. caballi*, and *Theileria equi* parasites in vitro cultures by using the half-maximal inhibitory concentration (IC50) values, their results appears that IC50 value of *Babesia* sp. as follow (53.3, 98.6, 30.1, 43.7, and 90.1 μm), respectively, these results assumed that this drug has a potential to be an alternative medication for piroplasmosis treatment [15,12], they are try the experiments to reduce clinical malaria transmition in children (2712 participants) through tested the ivermectin drug to village residents, all participants taken a single oral dose of this drug (150–200 μg/kg) at 3-week period, primary consequence was accumulative incidence of un-complicated malarias for 18 weeks, the refined using of IVM over the transportation seasons of malaria parasites can lessen its occurrence among children with no significant side effects on them.

Also [75], they evaluate the influence of IVM drug on soil worm in Ethiopia to eliminate soil transmitted helminths, 188 individuals were positive for any of the soil transmitted helminths species from 491 study participants, long-term of ivermectin drug reduced the prevalence of soil transmitted worm.

The effective of ivermectin drug seems to be assigned to a its persuades chloride-dependent membrane hyperpolarization, boosting the apoptosis process, as well as it considered as a highly powerful inhibitor of replication cell of pathogens [13], also ivermectin drug is trigger the releasing of the inhibitory neurotransmitter, gamma-aminobutyric acid from pre-synaptic nerve terminals, so it is repress signal transmission of ventral cord interneurons to excitatory-motor nerves in nematode; in fact, this drug couldn’t easily penetrate mammals’ central nervous system, so doesn’t interference with mammalian neurotransmission based on gamma-aminobutyric acid-dependent [16,13], or it is involving the inhibitory impact on Ribonucleic acid replication, hindering the binding to receptor sites, along with the immunomodulatory influence [76] , generally, these findings supports the encouragement to propose that it could be swiftly put into clinical experiments [13].

Ivermectin drug has a wide antiparasitic spectrum impact that is accomplished via the hyper-polarization of cell membrane, and consequently parasite paralysis and death [76,12], it was used for treating of multiple parasitic disease, such as strongyloidiasis and onchocerciasis [77,78], ascariasis , scabies , cutaneous larva migrans [79], filariasis , by its high efficacy and safety applications. Its wide therapeutic benefit led to its inclusion in the 21st vital medications [76],ivermectin medication was approved as antihelminthic, and has a wide spectrum activity toward parasitic nematodes as well as ectoparasitis, high vigor, and a comparatively long pharmacy-kinetic constantly in the circulation [12,78], it is an eclectic positively allosteric modulator for channels of glutamate-gated chloride in roundworm or threadworm and insects, through its binding with gated chloride channel which leads to chloride influx, hence hyper-polarization of cells and consequence loss function [80], Ivermectin has a capacity to kill a board range of parasites and vectors [15,16].

GC-MS is a procedure which integrate property of gas-liquid chromatography with mass spectrometry to recognize various substances in a specimen as quantity and quality [28,27], GC-MS applications involve medication detection, fire inquiry, environmental analysis, bomb investigation, and recognition of obscure samples, GC/MS can used to recognize a trace elements in substances that were formerly thought to have break up beyond identification [34,81and 27], oil of clove possess a huge number of benefits in medicine line, food flavoring, perfume, cosmetics and pharmaceutical manufacturing [82,83].

The results of GC-MS technical in present study revealed that terpenoid compounds of *S. aromaticum* fruit have many compounds where the retention time of terpenoid similar to time of stander compounds that found in the report of GC-MS library as found in table (4) and figure (1), current findings were agree with finding of [83] in Indonesia they determine the significant difference of constituents of clove oil ,they were isolated from clove bud specimens by steam distillation and the oil were analysis by GC-MS, this finding appears principle classes of compounds as eugenol , eugenyl acetate , caryophyllene also the results of present work similar to finding conducted by [84], they isolates oils from the distillation of clove and identify chemical compounds in essential oils, where they analyzed the sample using GC-MS method, the fimdings showed that a chemical compounds that identified are caryophyllene , phenol, 2-methoxy, caryophyllene and eugenol , as well as the findings of present work agree with [55], findings, they were point out the chemical components of flowers as well as flower stalks of cloves, the chemical components are analysis by GC-MS method which confirmed that the main component of clove is eugenol , caryophyllene ,phenol and non-phenolic compounds.

Its applications encompass evolution of a novel pharmaceuticals and examination of its purity, revelation of chemical warfare agent, inspection of athletes' urine, along with analyzing soil specimens [84], where *S. aromaticum* were inspected and phyto-chemical active groups were achieved, clove oil contains a distinctive active compound well known as eugenol [48], caryophyllene, and -humulene [85], eugenol is a principle vital compound, account for at least 50 percent, the residual from 10 to 40% is composed of eugenyl acetate, caryophyllene, and humulene, and a smaller amount, about 10% or less including a minor components like diethyl-phthalate, caryophyllene oxide, cadinene, copaene, 4--(2-propenyl)--phenol, chavicol, as well cubebene [86,85], finally, clove were successfully used for malaria, cholera, tuberculosis and neumerous of parasites that engaged with illnesses for human [48].

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The conclusion:

The present *In Vitro* study found that hot water extract of *S. aromaticum* flower buds possess a highly impact on paralysis as well death rate of *E. vermicularis* versus to cold water extract. Also,

terpenoid compounds of this extract with (150 µ/ml) concentration possess high effective impacts on the development of paralysis state as well as death of worm, followed by alkaloid compound and phenolic compounds, respectively. Using GCMS analysis, the current study proved that clove extract contains various compounds have biological activity like eugenol, eugenyl acetate, caryophyllene, and humulene.

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