

## Phytochemical and some pharmacological activity of acetone extracts of some Sudanese plants

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### Abstract

The *Trigonella foenum graecum* (TFG) and *Artemisia annua* (Aa) having medicinal value in Sudan for its use in gastrointestinal and inflammatory disorders. This study rationalizes the medicinal use of *Trigonella foenum graecum* and *Artemisia annua* separately and traditionally prepared combination of them as antispasmodic, antimicrobial, antioxidant. The antispasmodic effect was evaluated using rabbit jejunum preparation while the antioxidant activity was evaluated using DPPH method and the antimicrobial activity was studied against standard strains using paper disc diffusion method. The result was exhibited that Ethyl acetate, Acetone, Aquatic ethanol extracts of processed combined drug were induced appreciable relaxant effect on rabbit jejunum, Acetone extract of processed combined drug showed high activity against *Pseudomonas aeruginosa* at concentration 100 mg/ml with MIZD (31 mm). High antioxidant activity was seen with Acetone extracts of *A.annua* and *T.foenum graecum*.

**Keywords:** *Trigonella foenum graecum*, *Artemisia annua*, Antispasmodic, Antimicrobial, Antioxidant.

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## INTRODUCTION

Gastrointestinal smooth muscle spasm is the major symptoms of different gastrointestinal disorders. Much research has been carried out in elucidating the underlying mechanism causing motility disorders of the gut. The plants and plant products are frequently used as drugs, especially in developing countries[1]. Smooth muscle spasms are among causes that adversely affect the life quality. Antispasmodic agents like Hyoscine could be used. Fenugreek (*Trigonella foenum-graecum L.*) an annual crop belonging to the family fabaceae, about 30-60 cm tall and cultivated throughout the country. The plant contains alkaloids, glycoside, polyphenols, steroids, amino acids and volatile components. It is used as anti-diabetic, anti-fertility, anti-microbial. In India, it was used for lactic stimulation, indigestion and baldness. Recently it is also used as anti-diabetic, anti-cancer, immunomodulatory and anti-ulcer[2]. *Artemisia annua* is an Annual plant originated from China and grows mainly in the middle, eastern and southern parts of Europe and in the northern, middle and eastern parts of Asia. The aerial parts have been used in traditional Chinese medicine for the treatment of malaria, fever caused by tuberculosis, jaundice. *A.annua* has analgesic–antipyretic effects, antibacterial and anti-inflammatory activities. It consists mainly of essential oils, sesquiterpenoids, flavonoids and coumarins, proteins and steroids [3].

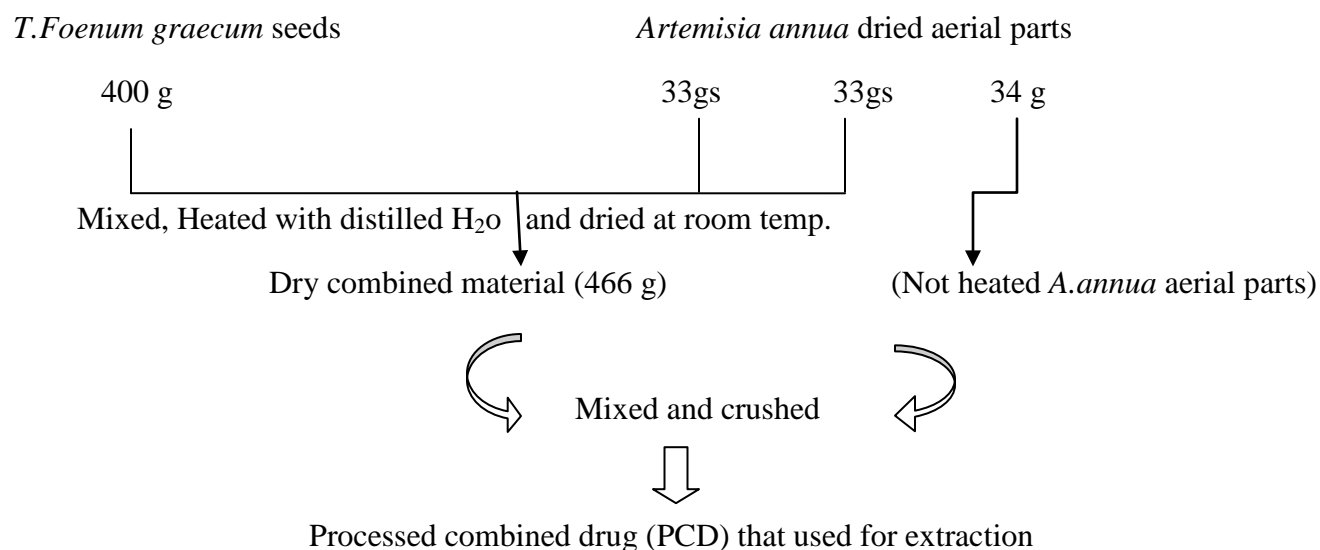
## MATERIALS AND METHODS

### Plant material

*Trigonella foenum-graecum L.* seeds and *Artemissia annua L.* areal parts were collected from Omdurman market as dry material during 2014, cleaned from dust and foreign matter then authenticated by the Medicinal and Aromatic plants Research Institute, Khartoum, Sudan.

### Preparation of combined plant material:

Traditionally the plant materials were prepared as following (Fatima, 2014).



### Preparation of extract

A weight (50gms) of TFG, Aa and PCD were crushed and each crushed plant materials were exhaustively extracted using Soxhlet apparatus with different organic solvents in order of increasing polarity: Petroleum ether (PET), Ethyl acetate (EtOAc), Acetone (Act) and 70% ethano l(Etoh). Each extract was filtered and evaporated under reduced pressure using Rotary evaporator (Harborne, 1984). The different extracts were preserved in refrigerator for further studies.

### Evaluation of Anti spasmodic activity of extracts

All extracts were screened for antispasmodic activity using standard method [4].

### Preparation of Tyroids solution :

The composition of Tyrode`s solution as[ NaCl (8g)+ KCl (0.2g) + CaCl<sub>2</sub>.2H<sub>2</sub>O (0.2g) + MgCl<sub>2</sub>. 6H<sub>2</sub>O (0.1g)+ NaH<sub>2</sub>PO<sub>4</sub> (0.05g)+ glucose (1g)+ NaHCO<sub>3</sub> (1g)]/ 1000ml distilled H<sub>2</sub>[4].

### Preparation of stock solution of Acetylcholine

Stock solution of Ach was prepared in concentration of 10 mg/100ml. one serial dilution was done by addition of Tyrode`s sol. to 1ml Ach (in ratio 1:9)[4].

### **Preparation of stock sol. of Atropine**

Stock solution of atropine was prepared in concentration of 10 mg/100ml. Two serial dilution was done by addition of 9 ml of Tyrode`s sol. to 1 ml of atropine stock sol. (stock sol. I, conc.0.01 mg/ml ), 1ml from the stock sol. I was added to 9 ml of Tyrode`s sol. (stock sol. II, conc.0.001 mg/ml ) [4].

### **Preparation of stock sol. of crude extract**

Stock sol. of crude extracts was prepared in Tyrode`s sol. in concentration of 1g/100ml.

### **Animals used**

Rabbits of 1-1.5 kg body weight of either sex were obtained from Omdurman market was used in the test. The rabbits were fed grass and water and adapted in standard laboratory condition before experiments.

### **Experimental setup**

The rabbit was fasted overnight and was killed by dislocating the neck, the abdomen was opened and suitable length of the rabbit jejunum was isolated and transferred to a beaker containing Tyrode`s sol. with aeration and cleaned from attached connective tissues then a piece of the rabbit jejunum (1-1.5 cm) was suspended in an organ bath containing Tyrode`s sol. with gut volume (GV) 50 ml according to the method of Magnus (1904), temp. was maintained at 37°C and the tissue left to equilibrate for 30 minute. Isotonic contractions of the longitudinal muscle were recorded using a Harvard Universal Kymograph recorder. A non cumulative dose response curve was first constructed for Ach at constant conc. (0.01 mg/ml) with doses 0.05 ml, 0.1 ml, 0.2 ml, 0.4 ml, 0.8 ml, 1.6 ml, 3.2 ml and a sub maximal dose (S.M) was chosen. The isolated rabbit jejunum was then subjected to tested extract at the same dose range used in Ach dose response curve (at constant conc. 10 mg /ml, GV 50 ml) each dose followed by Ach sub maximal dose to determine the active antispasmodic extract and to compare it with standard antispasmodic agent (atropine). The dose response curve of Atropine was constructed in the same way as that of extract at conc. (0.001 mg/ml). The jejunum was subjected to each tested dose of extract or atropine for a lapse of 1 min followed by (without washing) addition of the sub maximal dose of Ach with contact time of 30 second. Results were expressed as percentage of inhibition of control (Ach) response obtained before addition of extracts. In addition, the contraction of smooth

muscle of the rabbit jejunum preparation induced by Ach sub maximal doses when given alone were compared with that obtained when Ach was given in the presence of tested extracts.

### **Evaluation of anti-microbial activity**

#### **Preparation of standard bacterial suspensions**

One-ml aliquots of a 24-hours broth culture of the standard organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24 hours. The bacterial growth was harvested and washed off with sterile normal saline. The suspensions were stored at 4°C until used [5].

#### **Preparation of standard fungal suspensions**

The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25 °C for three days. The fungal growth was harvested and washed with sterile normal saline and the suspensions were stored at 4°C until used [5].

#### **Testing for Antibacterial Activity**

The paper disc diffusion method was used to screen the antibacterial activity of plant extracts and performed by using nutrient agar (NA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Crude extract sol. was prepared in methanol at conc. 100mg/ml. Bacterial suspension was diluted with sterile physiological solution to  $10^8$ cfu/ ml (turbidity = McFarland standard 0.5). 100 µl of bacterial suspension were swabbed uniformly on surface of the nutrient agar and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No. 1,6 mm in diameter) were placed on the surface of the NA and soaked with 20 µl of a solution of each plant extract. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The mean inhibition zone diameters (MIZD) were measured (mm).

### **Evaluation of anti-oxidant activity**

Antioxidant activity was determined in accordance with [6] with some modification. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activities of solution of the plant extract were determined. In 96 wells plate, the test samples were allowed to react with DPPH radical for 30 min. at 37° C. The conc. of DPPH was kept as (300µM). The test samples were dissolved in DMSO while

DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated control group. Propyl gallate (2mg/100µl) used as positive control. All tests and analysis were run in triplicate, Inhibition % was calculated according to equation:

$I \% = [(A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100]$ , Where  $A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except the extract),  $A_{\text{sample}}$  is the absorbance of the test compound.

### Statistical analysis:

Data analyzed using Excel sheet 2007.

## RESULT AND DISCUSSION

### Results

#### Antispasmodic activity

Antispasmodic potential of all tested extracts of the three drugs (expressed as inhibition % of control) was shown in Table (1) and Figure (1), that of all tested Acetone extracts was shown in Figure (2) and that of all tested Aquatic ethanol extracts was shown in Figure (3).

**Table (1): Maximum inhibition % of control of all tested extracts (conc.10mg/ml, GV 50ml)**

Crude drug	Extractive solvent	% Maximum inhibition	Maximum dose /ml
<i>T.foenum graecum</i>	Act	12.5%	1.6 ml
	Etoh 70 %	28.6%	1.6 ml
<i>A.annua</i>	Act	64.7%	3.2 ml
	Etoh 70 %	28.2%	3.2 ml
Processed combined drug	PET	-9.4%	3.2 ml
	EtOAc	89.5%	3.2 ml
	Act	100%	3.2 ml
	Etoh 70 %	83.3%	3.2 ml
Atropine		100%	0.2 ml

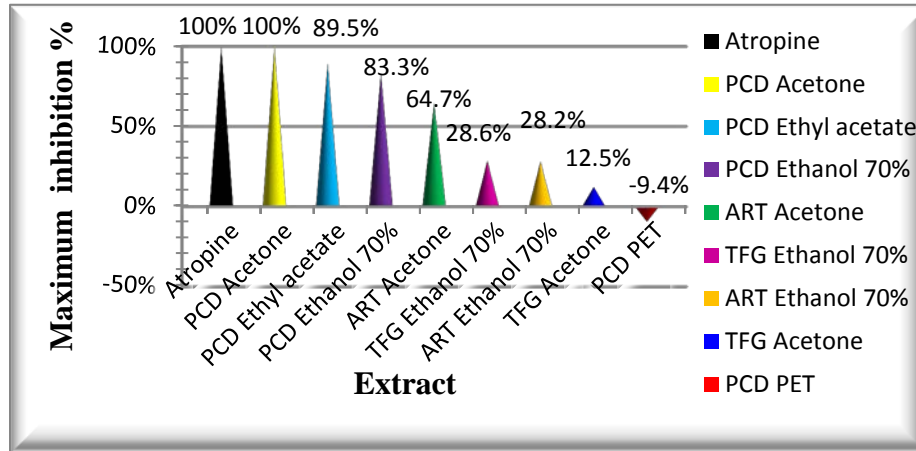


Figure (1): Maximum inhibition % of control of all tested extracts (at conc.10mg/ml, GV 50ml).

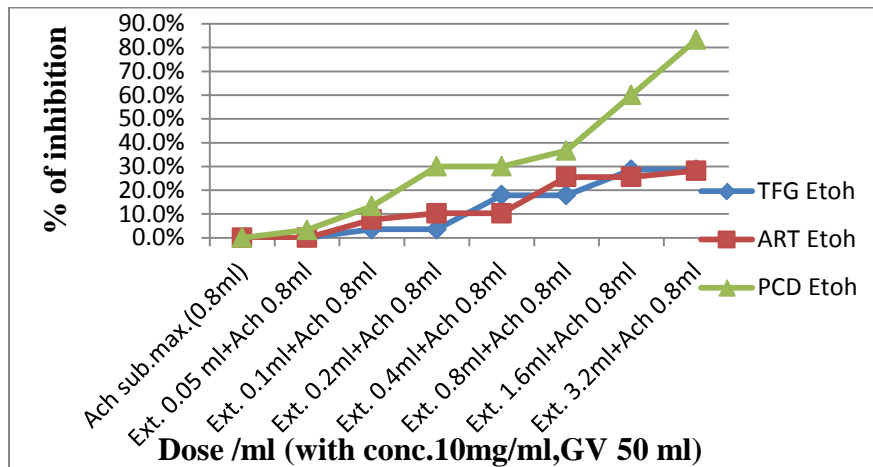


Figure (2): Comparison of relaxant effect of Aquatic ethanol extracts of TFG, Aa and PCD on Ach induced contraction of rabbit jejunum.

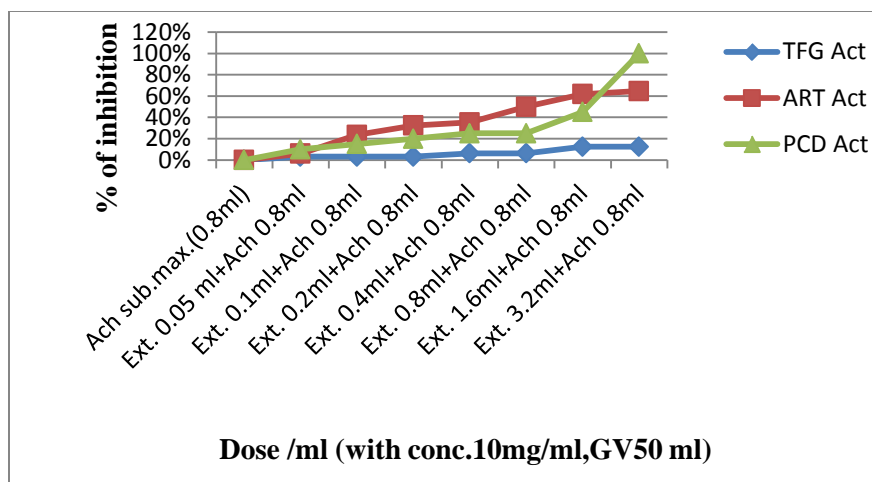


Figure (3): Comparison of relaxant effect of Acetone extracts of TFG, Aa and PCD on Ach induced contraction of rabbit jejunum.

Table (2): Antioxidant activity and Antimicrobial activity of tested extract against *E.coli* ( E.C), *Pseudomonas aeruginosa* (PS.) , *Staphylococcus aureus* (S.A), *Bacillus subtilis* (B.S), *Candida albicans* (C.A) and *Aspergillus niger*(A.N).

Crude drug	Extractive solvent	Antimicrobial activity						Antioxidant
		MIZD(mm) for bacterial strains ( conc.100mg/ml)				MIZD(mm) for Fungal strains (conc.100mg/ml)		% RSA ± SD (DPPH)
		E.C	PS.	S.A	B.S	C.A	A.N	
<i>T.foenum gracum</i> (TFG)	Act	20	21	13	13	13	14	80 ± 0.01
	Etoh 70 %	0	9	9	11	11	9	40 ± 0.1
<i>A.annua</i> (Aa)	Act	22	-	11	12	12	13	77 ± 0.02
	Etoh 70 %	18	15	18	15	16	16	56±0.19
Combined drug(PCD)	PET	-	-	-	-	-	-	28±0.4
	EtOAc	8	10	11	10	10	10	52.8±0.05
	Act	15	31	11	13	15	13	32 ± 0.09
	Etoh 70 %	11	-	-	-	-	7	51±0.08
Propyl galate (PG), standard Antioxidant								87.7±0.15



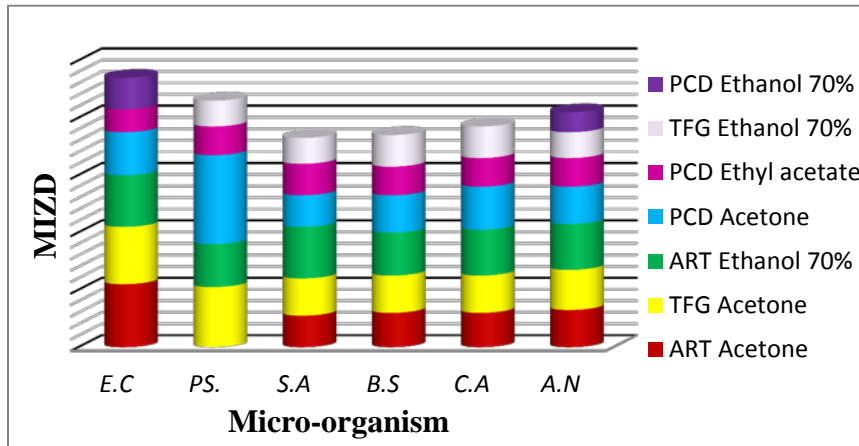


Figure (4): Comparison between Antimicrobial activities of tested extracts at conc.100mg/ml against E.C, PS. , S.A, B.S, C.A and A.N.

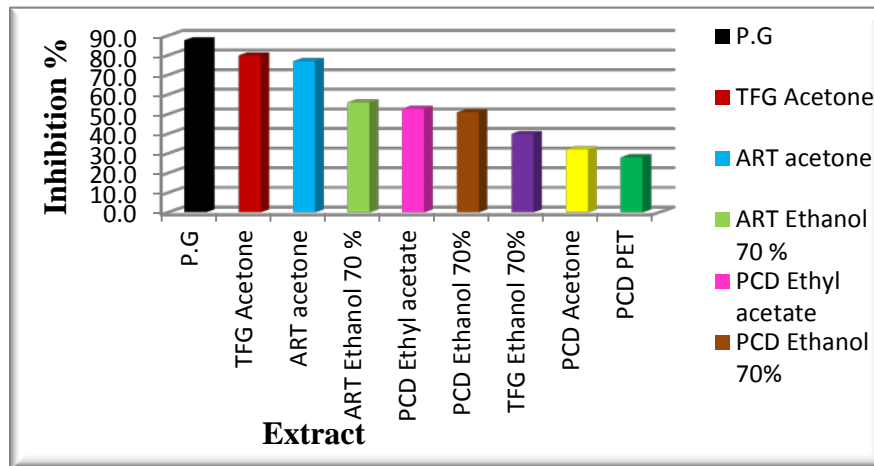


Figure (5): Comparison of Radioactive Scavenging activities of tested extracts and PG.

### **Antimicrobial activity**

Antimicrobial activity of all tested extracts from TFG, Aa and PCD was shown in Table (2) and comparison between them was shown in Figure (4). The mean inhibition zone diameters (MIZD) in mm of tested extract around each disc were measured.

### **Anti-oxidant activity**

Radioactive scavenging activities of all tested extracts and standard antioxidant (PG) were shown in Table (2), comparison between them were shown in Figure (5).

## **DISCUSSION**

All extracts evaluated on isolated rabbit jejunum preparation for possible antispasmodic activity. It was observed that Ach alone at sub maximal doses causes contraction of rabbit jejunum but when Ach was given in the presence of tested extracts there was reduction in response of jejunum to Ach that vary from extract to another reaching maximum with PCD Act extract, with no reduction was seen with PCD PET extract. Among tested extracts screened for possible Antispasmodic activity at conc. of 10mg/ml (GV 50 ml) with different doses, it has been found that the highest relaxant activity on rabbit jejunum was seen with the PCD Acetone extract (conc.10mg/ml, GV 50 ml) that started at dose of 0.2ml to reach maximum at 3.2ml (100% inhibition) followed by PCD Ethyl acetate extract (89.5% maximum inhibition at 3.2ml) and PCD Aquatic ethanol extract (83% maximum inhibition at 3.2ml). It was found that the Act and EtOAc extract of PCD exhibited a significant depressive effect on the non cumulative dose response curves of Ach and Also Pretreatment with atropine abolished the contractile effect of Ach. PCD PET extract showed little constant increment on rabbit jejunum response to Ach (about 9.4%) at doses range from 0.05 ml to 3.2ml (conc.10mg/ml) that may indicative for the presence of phytochemicals that have Ach like structure or Acetylcholinesterase inhibitory activity. The effect of all tested extracts was reversible after washing the tissue with Tyrode's solution. While relaxant activities of Etoh extracts of TFG and Aa were found to be close to each other (28.6, 28.2 % max. inhibition at 3.2ml respectively), Act extract of Aa was found to be of higher relaxant activities (64.7% max. inhibition at 3.2ml) than Act extract of TFG (12.5% max. inhibition at 3.2ml) which put into account the possible spasmolytic activity of the unique phytochemical that characterized acetone extract of Aa, Artemisinin, a sesquiterpene of known Antimalarial activity, [7] investigated

Anticholinergic effects of artemisinin, on isolated guinea pig heart preparations and suggested that the anticholinergic action of artemisinin is mediated via inhibition of the muscarinic potassium channel and/or associated GTP-binding proteins.

Many study revealed that Acetone is good solvent for Retrieval of artemisinin from Aa of these [8]. This may be justification for higher relaxant activity observed with Aa Act extract. The observed spasmolytic activity of Acetone extract of PCD at dose 3.2 ml (at conc.10mg/ml, GV 50 ml) was as same as that of Atropine, standard Antispasmodic agent at dose 0.4ml (conc. $10^{-3}$  mg/ml). The enteric nervous system is coordinates gastrointestinal motility. Gastrointestinal motility is regulated by numerous mediators, mainly Ach, histamine, 5-hydroxytryptamine, bradykinins, prostaglandins, substance P and cholecistokinins which achieve their contractile effects through an increase in cytosolic  $Ca^{2+}$ . Acetylcholine, a neurotransmitter, is released by the parasympathetic nervous system, and plays an important physiological role in the regulation of gut movements. To clarify the possible underlying mechanism, one should investigate the influence of the extract on Ach, histamine,  $BaCl_2$  and KCl-induced smooth muscle contraction. The Ach induced contractions involve two different mechanisms coupled to muscarinic receptors. To specify the spasmolytic activity of tested extracts which did not happen in the present study, the effect on the contraction of rabbit jejunum preparation induced by  $BaCl_2$ , KCl and H should be tested in other studies .There is growing evidence that the spasmolytic effect of many extract is associated with the presence of phenolic compounds. Flavonoids are one of the most numerous and widespread group of phenolics in higher plants. Some of them inhibit intestinal motility in vitro. Quercetin produces relaxation in ileum contracted by KCl. Apigenin and luteolin inhibited the contractions of isolated intestine. These substances have been reported to exhibit calcium antagonist and anticholinergic activities. The key role of phenolic compounds as spasmolytic agents is emphasized in several reports. Mulatu and Palacios-Espinosa have reported the spasmolytic activity of other plants species belonging to the same (Asteraceae) family[9]. The spasmolytic activity of the ethanol extracts of PCD studied here could be attributed to flavonoids and other phenolic compounds.

Complications seen with most antibiotics like hypersensitivity, GIT upset, in addition to antibiotic resistance made many researchers to seek about the plant of antimicrobial activity. Aa and TFG were studied widely for their antimicrobial activity which approved in several studies. The antimicrobial activity of essential oil from Aa was evaluated against representatives of gram-positive, gram-negative bacteria and fungi, using the agar diffusion method. All tested microorganisms were inhibited by

essential oil of *A. annua* (*Haemophilus influenzae*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Micrococcus luteus* and *Candida krusei* microbial strains)[10]. Bacterial growth can be inhibited by phytochemicals through several mechanisms. These plant products can act on various biochemical targets on the bacterial cells. The mode of action of phytochemicals is not completely understood, the chemical structure and properties influence the site of action of phytochemicals. The mechanism of action of essential oils against bacteria involves membrane disruption through the lipophilic structure. Alkaloids, such as berberine and piperine, interact with bacterial cytoplasmic membrane, intercalate with DNA or inhibit efflux pumps. Phenols act by interruption of energy production due to enzyme inhibition by the oxidized products, which react with sulfhydryl groups or non-specific interaction with proteins. In the case of flavonoids, they inhibit the synthesis of nucleic acids of gram-negative and gram-positive bacteria. Other authors, shown that glycoside saponins are able to induce pore-like structures that change the membrane permeability, they can also interfere with energy metabolism[11]. In this study, very high antibacterial activity was seen with PCD Acetone extract against *P.aeruginosa* that may be due to high content of artemisinin and polyphenols. According to [12] the acetone wextract of TFG seeds showed effective antimicrobial activity against *E.coli* and *Proteus vulgaris*, the phytochemical analysis in the mentioned study showed the presence of flavonoids, phenols, tannins, terpenoids, saponins and proteins and the study attribute the antimicrobial activity to individual or combined effect of the above mentioned Phytochemicals. While the PCD Act extract had higher inhibitory activity against *P.aeruginosa* than each individual drug, PCD Act extract had lower inhibitory activity against *E.coli* than each individual drug that may effect of elevated temp. used in processing , synergistic in case of *P.aeruginosa* or antagonistic in case of *E.coli*. Free radicals are implicated for more than 80 diseases including diabetes mellitus, atherosclerosis, cataract, rheumatism and other autoimmune disease like aging. In treatment of these diseases, antioxidant therapy has gained an utmost importance. Current research is now directed towards finding naturally occurring antioxidant of plant origin [13]. It is well known that oxidative stress or free radical damage has been implicated in the pathogenesis of cancer. The human body has evolved several potent antioxidant systems to mitigate the deleterious effects of reactive oxygen species and free radicals. Diet is a source of several important antioxidants. Herbs, fruits, vegetables and other dietary components have natural antioxidant property, thereby conferring medicinal properties to diet. Polyphenols compounds like flavonoids exert antioxidant activity by scavenging free radicals. Plant derived antioxidants are less likely to produce the side effects. Antioxidant property of *Trigonella foenum*

*graecum* seeds can be attributed to the presence of polyphenolic compounds such as narigenin, quercetin and coumarin. Flavonoids like vixetin, tricin, naringenin, quercetin and tricin 70 beta glucopuranoside present in *T.foenum graecum* seeds are good metal chelators and possess hydroxyl groups which are the ideal structural components for scavenging free radicals by hydroxyl ion donation, thereby contributing to the reducing power ability and radical scavenging activity exhibited in our study, Flavonoids also exert antioxidant property by inhibition of lipid peroxidation, platelet adhesion and aggregation. Saponins could possibly contribute to the antioxidant property by inhibition of lipid peroxidation[14]. Clearly indicated by [15] that both ethanol extract of Fenugreek and its major alkaloid, trigonelline are promising natural antioxidants Furthermore, the nicotinic acid, one of the metabolite of trigonelline, also had an antioxidant effect. Many studies were conducted to investigate the antioxidant activities of *A.annua* aerial parts, these studies approved that this plant has good antioxidant activities due to high polyphenols content. However, another study conducted by [10] mentioned that the major constituents of essential oil of *A.annua* were oxygenated monoterpenes, artemisia ketone and camphor, they Isolated essential oil and tested it for radical-scavenging ability using the stable DPPH assay among other tests and they concluded that in all tests oil did not show a prominent antioxidant activity. This may justifies the low RSA% of PCD PET extract ( $28\pm 0.4$ ), another justification may be the method of processing and extraction that depend on heating that may lead to loss of essential oils. Generally, phenolic compounds account for most of the antioxidant activities in plants. [16] Reported that Terpenoids such as triterpenes, sesquiterpenes and diterpenes have been referred to as antibiotics, insecticidal, anthelmintic and antiseptic in pharmaceutical industry, Alkaloids possess analgesic, antispasmodic and bactericidal, antimalarial and analgesic activities and Proteins act as antibiotic and antimicrobial agents. The two basic plants used in this study, TFG and Aa known to be of high contents of different phytochemicals of high medicinal value. Artemisinin, a sesquiterpene in Aa, Trigonelline, an alkaloid in TFG and Protins, flavenoids, essential oils , coumains, saponins and other polyphenols all were found to be in both plants by several studies and could be affected positively or negatively by elevated temp. used in processing and extraction, Moreover, many synergistic or destructive chemical reactions may takes place between the phytochemicals of the two plants when combined especially upon heat processing and soxhlet extraction used in this study.

## CONCLUSION

In the present study, from all observations and results obtained it was concluded that the Acetone, Ethyl acetate extracts of processed combined *Trigonella foenum graecum* with *A.annua* and Acetone extract

of *A.annua* alone have good relaxant activity on isolated rabbit jejunum and accordingly exhibits promising anti-spasmodic activity that when compared with a standard anti-spasmodic agent (atropine), it was found to be comparatively less potent than atropine and as a result of many problems seen with antispasmodic agents like urine retention and dry mouth, using a drug of a herbal origin with good antispasmodic activity but with high degree of safety and efficacy could be a suitable alternative. Toxicity and clinical studies of processed combined drug of TFG and Aa should be carried. Furthermore, Acetone extract of PCD showed excellent Antibacterial activity against *P.aeruginosa* and good one against *E.coli* which may be of great value in treating intestinal spasm of *Pseudomonas* or *E.Coli* infestation origin. The high Antioxidant activity seen with TFG and Aa Acetone extracts and by contrast lower one of PCD may put on the table the negative effect of heat processing suggesting another study to investigate the Antioxidant activity of unprocessed combination. On this area, many studies to evaluate Anticancer activity of PCD and other qualitative and quantitative studies to investigate the phytochemicals founded in PCD should carried out to justifies variation in biological activities of the three drugs, *T.foenum graecum*, *A.annua* and their processed combination.

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