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EVALUATION OF THE ANTIULCER ACTIVITY AND GC-MS SPECTROSCOPIC ANALYSIS OF THE CRUDE ETHANOLIC EXTRACT OF PEURARIA PHASEOLOIDE LEAF (ROXB) BENTH. (FABACEAE)

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ABSTRACT

The current study was carried out to evaluate the antiulcer activity using pylorus-ligation induced model and a GC-MS analysis of the ethanolic leave extract of *Peuraria phaseoloides* plant. Gastric protection was evaluated by assessing different parameters like gastric volume, pH, ulcer index and percentage inhibition of ulceration. Omeprazole was used as the standard at a dose of 20mg/kg while those of the extracts were 50mg/kg, 150mg/kg and 300mg/kg. The extract showed better activity when compared to the negative control. Significant reduction in ulcer index (measures of ulcerated area) was noted for 50mg/kg ($_P$<0.01), 150mg/kg ($_P$<0.05) and 300mg/kg ($_P$<0.01) as compared to the negative control. A total of 15 bioactive compound were characterized through the GC-MS analysis of the ethanolic leave extract. The present study indicates that ethanolic leave extract of *Peuraria phaseoloides* are of great medicinal value and in turn can serve as a data source for the development of many therapeutic agents.

KEYWORDS: *Peuraria phaseoloides*, pylorus ligation, GC-MS, antiulcer.

INTRODUCTION

In recent years, demand for identification and evaluation of new drugs probably of plant origin are gaining popularity for treating diseases. In the same spirit, Maesen, 1985, discovered that tuberous roots of *Pueraria phaseoloides* which are edible, a decoction of the plant can be used as an anti-infective agent and a poultice applied to ulcers and boils especially in children. Moreover, in Southern part of Nigeria, Pueraria phaseoloides leaves are often crudely used for the treatment of ulcer (Okoye et al., 2020). However, to the best of our knowledge, there is scanty or no scientific studies which had been reported about the leaf of the plant or any extractions from its leaf used internally as an antiulcer agent. Therefore, there is need to cover this lacuna in the literature which this study sets to achieve. Hence, the study is specifically set to evaluate the anti-ulcer activity of the crude ethanolic extract of *Pueraria phaseoloides,* which is attributed by the presence of phytochemicals inherent in the plant extract and also to determine the GC-MS spectra of the plant extract, as it equally helps to reveal the (most) active component(s)of the plant as well as their biological activities which are utilized majorly for their therapeutic purposes. The work is structured as follows: Part one contains the introduction, part two has the theoretical underpinning and literature review and part three contains methods while part four is results and findings, part five is discussion. Then finally is the implication to research and practice, conclusion and future research.

Literature/Theoretical underpinning

Ulcers are an open sore that develop in the lining of the stomach, lower oesophagus or small intestine. Ulcers are lesions on the surface of the skin or a mucous membrane characterized by a superficial loss of tissue. Ulcers are most common on the skin of the lower extremities and in the gastrointestinal tract, although they may be encountered at almost any site. There are many types of ulcer such as mouth ulcer, oesophagus ulcer, peptic ulcer, and genital ulcer, these peptic ulcer is seen among many people. The two most common types of peptic ulcer are called “gastric ulcer” and “duodenal ulcer.” The name refers to the site of ulceration. A person may have both gastric and duodenal ulcers at the same time. Gastric ulcers are located in the stomach, characterized by pain; ulcers are common in older age group. Eating may increase pain rather than relieve pain. Other symptoms may include nausea, vomiting, and weight loss. Although patients with gastric ulcers have normal or diminished acid production, yet ulcers may occur without acid secretions because they are regarded as wounds. Duodenal ulcers are found at the beginning of small intestine and are characterized

by severe pain with burning sensation in upper abdomen that awakens patients from sleep. Generally, pain occurs when the stomach is empty and relieves after eating. The pathophysiology of peptic ulcer disease involves an imbalance between offensive (acid, pepsin, and Helicobacter pylori) and defensive factors (mucin, prostaglandin, bicarbonate, nitric oxide, and growth factors) (Hoogerwerf and Pasricha, 2001).

Prevalence of peptic ulcer disease

Epidemiological studies show that PUD remains a relatively common condition worldwide, with annual incidence ranging from 0.10% to 0.19% for physician-diagnosed PUD and from 0.03% to 0.17% for PUD diagnosed during hospitalization. The data show that the incidence of PUD has decreased over recent decades, most likely as a result of the decrease in *H. pylori* infection, particularly in Western populations. However, it is possible that the situation may be different in Asian countries. The most reliable study of physician-diagnosed prevalence was from Sweden, reporting cross-sectional data representative of the general population, the study thus included both symptomatic and asymptomatic PUD. The overall prevalence of PUD observed in this study was 4.1%; 19.5% of all PUD cases identified were asymptomatic. Comparing this prevalence with the lower rates obtained from other studies of physician diagnosed PUD in primary care suggests that a proportion of individuals with PUD remain undiagnosed(Lüllmann *et al.*, 2000, Andersen et al., 2018).

Causes of peptic ulcer disease

No single cause has been found for ulcers. However, it is now clear that an ulcer is the end result of an imbalance between digestive fluids in the stomach and duodenum. Peptic ulcers are once believed to be caused by spicy food and stress; these have been found merely to be aggravating factors because study has revealed it to be caused as a result of a gram-negative bacterial infection known a (Helicobacter pylori). Factors that can increase your risk for ulcers include: use of painkillers called non-steroidal anti-inflammatory drugs and many others available by prescription. (Lüllmann *et al.*, 2000)

Symptoms and Complications of peptic ulcer disease

An ulcer may or may not have symptoms. When symptoms occur, they may include: a gnawing or burning pain in the middle or upper stomach between meals or at night, bloating, heartburn, nausea or vomiting. In severe cases, symptoms can include: dark or black stool, vomiting blood, weight loss, severe pain in the mid to upper abdomen. Though, ulcers often heal on their own, yet warning signs should never be overlooked (Lüllmann *et al.*, 2000).

The acute clinical complication of ulcers is bleeding and approximately occurs in 15%-20% of ulcers. Bleeding is also the leading cause of death in geriatric individuals. Chronic symptoms include ulcer perforation and stricture formation (Lüllmann *et al.*, 2000).

Diagnosis of peptic ulcer disease

A fundamental principle of specific antimicrobial therapy is accurate diagnosis. Numerous validated methods to diagnose patients with *H. pylori* infection are currently in practice as it represents one of the most chronic diseases of humans. The diagnostic methods are broadly classified as: invasive method and non-invasive method. (Andersen et al., 2018)

Invasive Tests include: Endoscopy and Histology

Non-invasive tests include: Serology and Urea breath test

MATERIALS AND METHODS

Plant materials

Pueraria phaseoloides leaves were collected in July 2019 from Nando in Oyi Local Government Area of Anambra State, Eastern Nigeria. The plant was identified and authenticated by a taxonomist in the Pharmacognosy and Traditional Medicine Department of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Nigeria. Herbarium specimen deposited with herbarium number PCG 474/A/030.

Animals

Wister Albino rats of either sex obtained from animal house of Veterinary Department of University of Nigeria, Nsukka. Weighing 150-200g each were used. Animals were maintained under standard husbandry conditions in the animal house, fed with water and a recommended rodent diet.

Equipments

Electronic digital weighing balance (Ohaus Corp, USA), water bath (Serological, England), beakers (Pyrex;10, 50, 100 and 1000 ml), measuring cylinders, hand grinding machine (Ohaus Corp, USA), syringes and needles (1, 2 and 10 ml capacity), refrigerator (Thermocool, England), cotton wool (Pyrex. Nig), Animal weighing balance (Ahus China), Well ventilated cage, Needle and thread, Glass funnel(Pyrex), Muslin cloth, Filter paper(Number 1 Whatman), Pestle and motar, Spatula-stainless steel, Test sieves, Micro-syringes, Vials, GC-MS with Purge and Trap.

Reagents and Chemicals

Ethanol (JHD), Distilled water (NBC, Nig), Ketamine (Esketamine, Nig), Omeprazole (Ometab INDIA), Anhydrous Sodium Sulphate, Methanol, Organic free reagent water.

Preparation of plant material

The leaves were thoroughly washed and shade dried at room temperature for two weeks. The dried leaves were pulverized with an analytical milling machine and sieved to control the particle size. Lastly, stored in an airtight container for further analysis (Bruce et al., 2016, Bruce et al., 2019)

Extraction

A quantity (600 g) of the powdered leaves was extracted using ethanol (2500 ml) with occasional stirring for 72hrs by cold maceration. The mixture was sieved using Muslin cloth and filtered with a filter paper. The filtrate was dried *in vacuo* at 40°C and extract was stored in a refrigerator for use (Onyegbule *et al*., 2019).

GC/MS Analysis

The Gas chromatography-mass spectrometry (GC–MS) analyses of *Peuraria phaseoloides* ethanolic leaf extract were carried out in Agilent Technologies (Wilmington, Delaware, USA) equipment with a column HP-5MS (30 m × 0.25 mm 1D X 0.25). A sample of 1 µL of each extract was injected. For detection, an ionization system with energy of 70 eV was used. The flow rate of carrier gas was constant and it was maintained at 1.1 mL/min. The injection temperature was 250 °C. The warming program of the oven was isothermal for 5 min at 60 °C followed by a warming of 5 °C/min up to 100 °C/min (2 min), and 10 °C/min up to 250 °C/min (5 min). The interpretation of the mass spectra was made using the National Institute of Standard and Technology (NIST) library. (Bruce et al, 2021; Gnanavel and Mary, 2013)

Acute toxicity test

Acute toxicity test Acute toxicity study was carried out using the limit test dose of 2000mg/kg as demonstrated by OECD 425 guideline (OECD guidelines 2008). Three albino rats were fasted for 24hours but were allowed to take water. A limit dose of 2000mg/kg of Peuraria phaseoloides was given accordingly and animals were monitored one after the other for behavioural profile, autonomic profile, neurologic profile, physical states, after which a steady drug administration for 2hours was carried out, during the first 24hours and on daily basis thereafter for a total 2weeks (Ihekwereme et al., 2020).

Pylorus ligation induced ulcers

The experimental animals were separated into five groups, where the first group served as the control and were administered distilled water (5ml/kg, *p.o*).The second group were administered omeprazole (20mg/kg *p.o*). Then the third, fourth and fifth group were administered 50mg/kg, 150mg/kg and 300mg/kg of the ethanolic plant extract respectively.

Prior to the pylorus ligation, the animals were starved of food for 48hours but were freely given water as much as they can take and were singly placed in their separate cages. Anaesthetized with 30mg/kg ketamine each, the abdomen was cut open by a small midline incision beneath the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed by interrupted sutures. The animals were denied of food and water during the postoperative session, after which the rats were sacrificed by a harmful dose of the anaesthetic agent (ketamine) after 6 hours of pyloric ligation. The abdomen was opened, cardiac end of the stomach was dissected out, and the contents were emptied into a glass tube. The volume of the gastric juice was measured after centrifugation at 2000rpm for 10mins. From the supernatant, a small volume (1ml each) were taken for the determination of pH and total acidity. Each stomach was examined for lesions in the forestomach portion and indexed according to severity.

Statistical analysis

The statistical analysis was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's Comparisons Test. The values were expressed as mean ± standard error of mean (SEM). $_P$>0.05 was considered statistically non-significant while $_P$<0.01 and $_P$<0.05 was considered statistically significant.

RESULTS

GC-MS Interpretation

Table 1

S/No	Area	Retention time (mins)	Compound name	Molecular formula	Molecular weight
1	6.38	6.010	Glycerin	$C_3H_8O_3$	92
2	5.44	6.169	Glycerin	$C_3H_8O_3$	92
3	1.77	6.786	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C_6H_8O	144
4	1.51	12.039	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
5	19.02	12.274	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256

6	10.06	12.421	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
7	4.29	13.192	Phytol	$C_{20}H_{40}O$	296
8	16.41	13.415	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278
9	16.22	13.521	Octadecanoic acid	$C_{18}H_{36}O_2$	284
10	2.91	13.710	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312
11	1.93	17.015	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330
12	4.99	24.027	Squalene	$C_{30}H_{50}$	410
13	3.39	32.361	Vitamin E	$C_{29}H_{50}O_2$	430
14	3.40	35.355	Stigmasterol	$C_{29}H_{48}O$	412
15	2.27	39.720	D:B-Friedo-B':A'-neogammacer-5-en- 3-ol, (3.beta.)	$C_{30}H_{50}O$	426

Table 2: Biological activity of identified compounds of *peuraria phaseoloides.*

Compound	Biological activities	References
Glycerin	Laxative, Antibacterial	
Glycerin	Laxative, Antibacterial	
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Antioxidant, Antimicrobial, Anti-inflammatory, antiproliferative, anticancer,	Kumar et al., 2010
Hexadecanoic acid, methyl ester	Antimicrobial, hepatoprotective, antioxidant, Antiallergic	Rukshana et al., 2017, Bruce et al 2021
n-Hexadecanoic acid	Antioxidant, Antiandrogenic, flavour, Pesticide, 5-Alpha reductase inhibitor, Hypocholesterolemic, Hemolytic, nematicide , lubricant	Omoregie et al., 2015
Hexadecanoic acid, ethyl ester	lubricant, antimicrobial, Hypocholesterolemic, Flavor, Cosmetic	Gideon V.A, 2015
Phytol	Diuretic, antimicrobial, anticancer, anti-inflammatory	Rajalakshmi and Mohan, 2016
9,12,15-Octadecatrienoic acid	Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge	Kavitha et al., 2014
Octadecanoic acid	Antimicrobial	Rahuman et al., 2000
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Hemolytic, Pesticide, Flavour, Antioxidant, Nematicide	Lalitha et al., 2015, Bruce et al.,
Squalene	Antibacterial, Antioxidant, Immunostimulant, Pesticide, Antitumor, Lipoxygenase-inhibitor	Zih-Rou et al., 2009
Vitamin E	Antispasmodic, Hepatoprotective,	Rajalakshmi and Mohan, 2016

	Anticancer, Anti-inflammatory, Antidermatitic, Antileukemic, Hypocholesterolemic, Analgesic	
Stigmasterol	Antiasthmatic, Antiarthritic, Antimicrobial, Antiinflammatory, Anticancer, Diuretic	Lalitha et al., 2015, Bruce et al., 2016
D:B-Friedo-B':A'-neogammacer-5-en- 3-ol, (3.beta.)	Unknown	

Table 3: Effect of crude ethanol extract of *P. phaseoloides* on ulcer Index and Percentage ulcer protection in pylorus ligation induced gastric ulcer.

S/N	Group	Treatment	Ulcer Index	% Ulcer Protection
1.	I	5ml/kg Distilled water	10.93±0.26	0
2.	II	20mg/kg Omeprazole	6.97±0.15**	36.23
3.	III	50mg/kg Crude extract	6.87±0.13**	37.12
4.	IV	150mg/kg Crude extract	10.2±0.03*	6.68
5.	V	300mg/kg Crude extract	6.90±0.12**	36.87

Values expressed as mean± SEM, n=5 in each group, statistical comparisons as follows: significant at P<0.01** and P<0.05* compared to control group (I).

Table 4: Effect of crude ethanol extract of *P. phaseoloides* on gastric fluid $_{P}$H.

S/N	Group	Treatment	$_{P}$H
1.	I	5ml/kg Distilled water	2.67±0.17
2.	II	20mg/kg Omeprazole	5.5±0.25**
3.	III	50mg/kg Crude extract	3.0±0.00**
4.	IV	150mg/kg Crude extract	3.5±0.29*
5.	V	300mg/kg Crude extract	3.0±0.00**

Values expressed as mean± SEM, n=5 in each group, statistical comparisons as follows: significant at P<0.01** and P<0.05* compared to control group (I).

Table 5: Effect of crude ethanol extract of *P. phaseoloides* on gastric fluid volume.

S/N	Group	Treatment	Volume of Gastric Fluid
1.	I	5ml/kg Distilled water	3.23±0.42
2.	II	20mg/kg Omeprazole	0.2±0.10**
3.	III	50mg/kg Crude extract	0.52±0.17**
4.	IV	150mg/kg Crude extract	0.82±0.25**
5.	V	300mg/kg Crude extract	0.91±0.26**

Values expressed as mean± SEM, n=5 in each group, statistical comparisons as follows: significant at P<0.01** compared to control group (I).

Table 6: Effect of crude ethanol extract of *P. phaseoloides* on number of ulcers.

S/N	Group	Treatment	Number of Ulcers
1.	I	5ml/kg Distilled water	8.0±2.25
2.	II	20mg/kg Omeprazole	2.67±1.33*
3.	III	50mg/kg Crude extract	1.67±0.20*
4.	IV	150mg/kg Crude extract	1.33±0.33*
5.	V	300mg/kg Crude extract	1.67±0.88*

Values expressed as mean±SEM, n=5 in each group, statistical comparisons as follows: significant at P<0.05* compared to control group (I).

Table 7: Effect of crude ethanol extract of *P. phaseoloides* on gastric fluid inhibition.

S/N	Group	Treatment	Gastric Fluid Inhibition (%)
1.	I	5ml/kg Distilled water	0
2.	II	20mg/kg Omeprazole	93.81
3.	III	50mg/kg Crude extract	83.9
4.	IV	150mg/kg Crude extract	74.6
5.	V	300mg/kg Crude extract	71.83

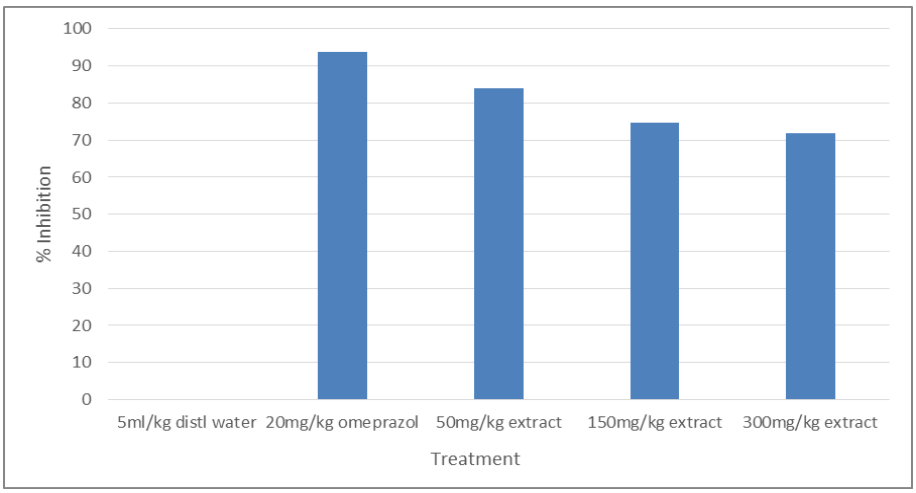


Fig. 1: Gastric acid inhibition.

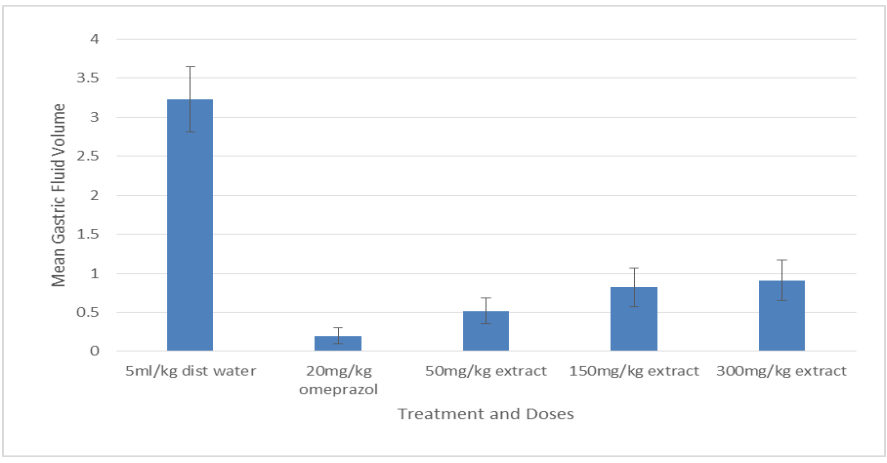


Fig. 2: Mean gastric fluid volume against doses.

DISCUSSION

Natural products are renowned source of phytochemicals that serve conventional therapeutic remedy for various diseases (Priyanga et al., 2014, Bruce and Onyegbule 2021). Many phytochemical evaluations have been done in various places in the world using GCMS over the years (Abirami and Rajendran, 2011; Gopalakrishnan et al, 2011; Sangeetha and Vijayalakshmi, 2011; Vanitha et al, 2011; Wu et al, 1010;). Gas chromatography combined with mass spectrometry (GC-MS) gives a more exact data for qualitative analysis (Cong et al, 2007). Graphically, the X-axis denotes the retention time in minutes of each compound while the Y-axis denotes the intensity/the presence of peak area at various retention. The compounds from the GC-MS investigation indicates the biological properties and in turn the pharmaceutical values of the study plant extract. Ethanolic leave extract of *Peuraria phasoeloides* on GC-MS analysis revealed; Glycerin, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Phytol, 9,12,15-Octadecatrienoic acid, Octadecanoic acid, ethyl ester, Hexadecanoic acid, 2-hydroxy-1-(hydroxyl-methyl) ethyl ester, Squalene, Vitamin E, Stigmasterol, D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.) as well as their corresponding biological effects described in table 3 above.

Hexadecanoic acid, ethyl ester is a fatty acid ester with nematicide, pesticide, lubricant, anti-androgenic, flavor, and has hemolytic 5-alpha reductase inhibitor features (Venkata-Raman et al., 2012; Aneesh TP et al., 2013). Also, n-Hexadecanoic acid generally regarded as Palmitic acid contains nematicide, pesticide, lubricant, anti-androgenic, flavor, hemolytic 5-alpha reductase inhibitor, antioxidant and hypo-cholesterolemic features (Komansilan et al., 2012). In a similar way, GC-MS studies of ethyl acetate extract of Goniothalamus umbrosus unveiled the presence of n-Hexadecanoic acid (Ibraham *et al*., 2009), Hexadecanoic acid, 9, 12 - Octadecadienoic acid, n-hexadecanoic acid, 9, 12, 15-Octadecatrienoic acid, Squalene and Phytol were present in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009), hence this is in agreement with the present study.

The aetiology of peptic ulcer is unknown in most of the cases, yet it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defence mechanisms. To regain the balance, different therapeutic agents are used to inhibit the gastric acid secretion or to boost the mucosal defence mechanisms by increasing mucosal production, stabilizing the surface epithelial

cells, or interfering with the prostaglandin synthesis (Mohod and Bodhankar, 2011). The phytochemical testing of *Pueraria phaseoloides* ethanol leaf extract revealed the alkaloids, saponins, tannins, flavonoids, terpenoids, cardiac glycosides, carbohydrates, and reducing sugars (Okoye et al., 2020). Similarly, ethanol releases more diverse phytochemicals such as anthocyanins, tannins, saponins, terpenoids, xanthoxylines, totarol, quassinoids, lectones, flavones, phenols, and polyphenols than other extraction solvents (Tiwari *et al.*, 2011). These secondary metabolites are effective as antioxidant, antineoplastic, anti-ulcer, anti-inflammatory, and immune stimulating agents (Mohod and Bodhankar, 2011; Paguigan *et al.*, 2014). Flavonoids are thought to increase mucosal prostaglandin content, decrease histamine secretion from mast cells by inhibition of histidine decarboxylase, inhibit *Helicobacter pylori* growth, act as free radical scavengers, and inhibit H^+/K^+-ATPase (Repetto and Llesuy, 2002). Saponins may activate mucous membrane protective factors, and tannins render the outermost layer of the mucosa less permeable, for instance, to chemical irritation (Borrelli and Izzo, 2000). In addition, terpenoids and alkaloid compounds are also reported to have potent activity against gastric ulcers (Mitra *et al.*, 2014).

Pylorus-ligation induced gastric ulcers occurs as a result of elevation in acid-pepsin accumulation because of pyloric obstruction and mucosal digestion. Large amount of mucus is secreted as a result of superficial damage and this in turn provides a favourable microenvironment in repair. Pylorus-ligation induced ulcer model is a method of peptic ulcer evaluation which studies the mean ulcer index in ulcerogenesis, which in this case may be stress induced secretion of HCL in a large quantity from the parietal cells and autodigestion of mucosa by the gastric juice (Mohod and Bodhankar, 2011; Sharath., *et al* 2015; Raju *et al*., 2009). Free radicals may also be associated since studies have shown changes in the antioxidant status following pylorus ligation-induced ulceration in rats. These factors are associated with the development of upper gastrointestinal damage including lesions, ulcers and life-threatening perforation and haemorrhage. Prostaglandin E2 and I2 are predominantly synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate. Hydrophobic surfactant-like phospholipids secretion in the gastric epithelial cells is also stimulated by the prostaglandin. Effect of pylorus ligation has caused the accumulation of gastric secretion. (Sharath *et al*., 2015). In the present study, different doses of the extract (50, 150 and 300mg/kg) were evaluated for their effect on volume of gastric secretion, pH, ulcer score and ulcer index along with the standard drug omeprazole (20mg/kg). The extract showed better activity when compared to the

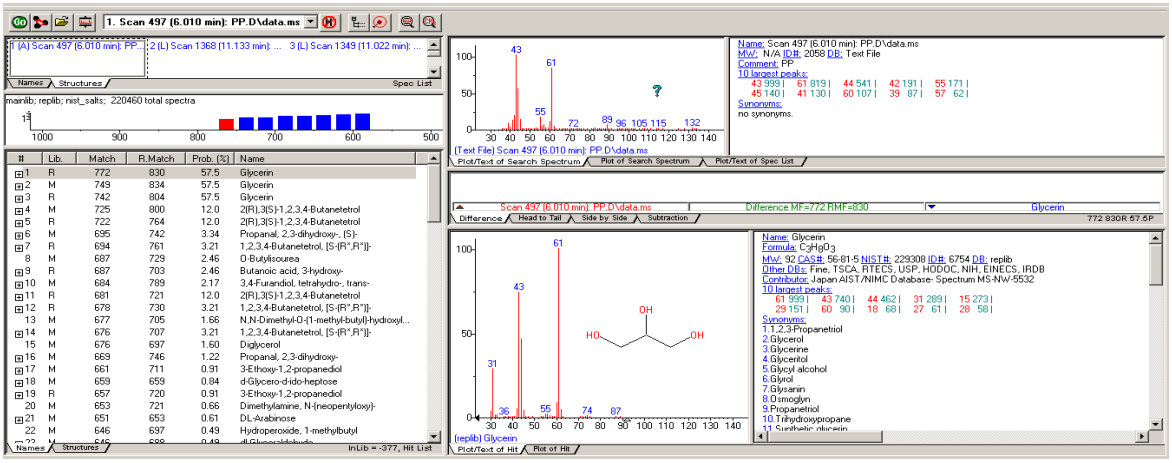
negative control. Significant reduction in ulcer index (measures of ulcerated area) was noted for 50 mg/kg ($P<0.01$), 150 mg/kg ($P<0.01$) and 300 mg/kg ($P<0.01$), 150mg/kg ($_P<0.05$) and 300mg/kg ($_P<0.01$) as compared to the negative control. While omeprazole 20mg/kg has more anti-secretory effect than all extract treatment groups (50, 150 and 300mg/kg), both 50mg/kg and 300mg/kg of extract amazingly exhibited a better reduction of ulcer index than the standard drug (ie 6.87±0.13 and 6.90±0.12 against 6.97±0.15), which might be due to more combined cyto-protective and anti-secretory activity effect of the extract than of the standard drug. Flavonoids are suggested to possess both these effects (Borrelli & Izzo, 2000). The better reduction in ulcer index noted for 50mg/kg and 300mg/kg of extract compared with the standard drug omeprazole (20mg/kg) is in line with a report by Melese *et al.* (2011) where the aqueous extract of *Plantago lanceolata* L. at a dose of 400mg/kg showed a better ulcer inhibition than the standard drug rantidine. Such findings strengthen the search for novel agents by tapping the rich herbal drugs used in folk medicine.

Findings from this study have shown that phytochemical constituents such as flavonoids, tannins and saponins present in this plant could be responsible for the observed anti-ulcerogenic anti-gastric activity. This is in agreement with a study conducted by Olaleye *et al*. (2008) which suggests that these constituents are responsible for anti-ulcer activity.

Implication to research and practice

From the just concluded study which evaluated an analytical test procedure (GC-MS analysis) together with the ulcerogenic findings, it was revealed that *Peuraria phaseoliodes* leaves are endowed with a lot of significant phytochemical possessing various biological activities. These important components will in turn serve as a data source in further studies (experiments) for researcher and scientist in different fields like; the food industry, pharmaceutical industry, chemical industry and many more as well as scholars in academics.

APPENDIX



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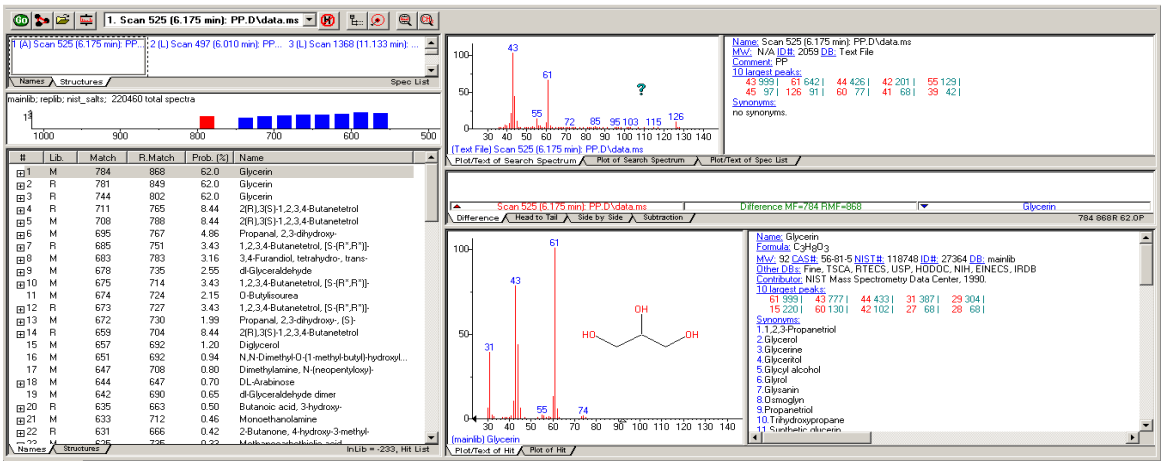

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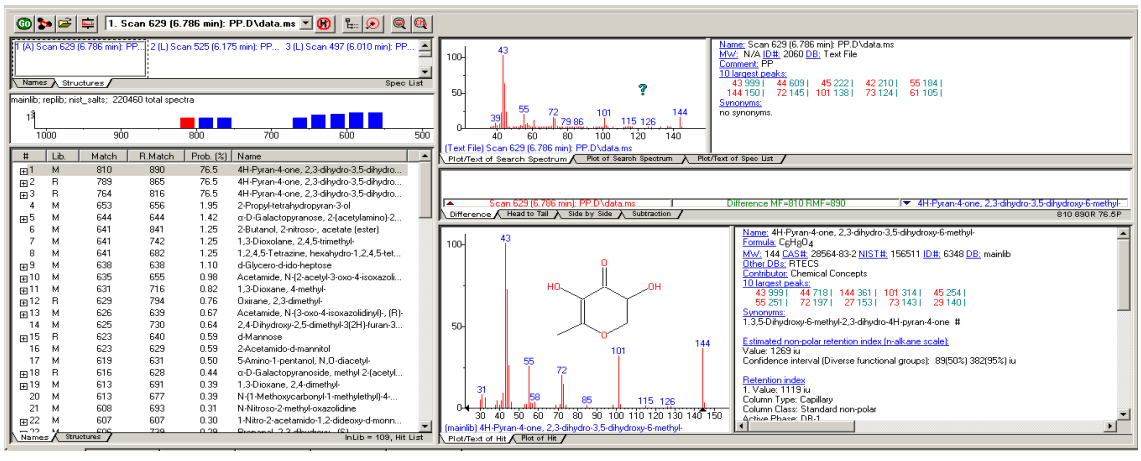

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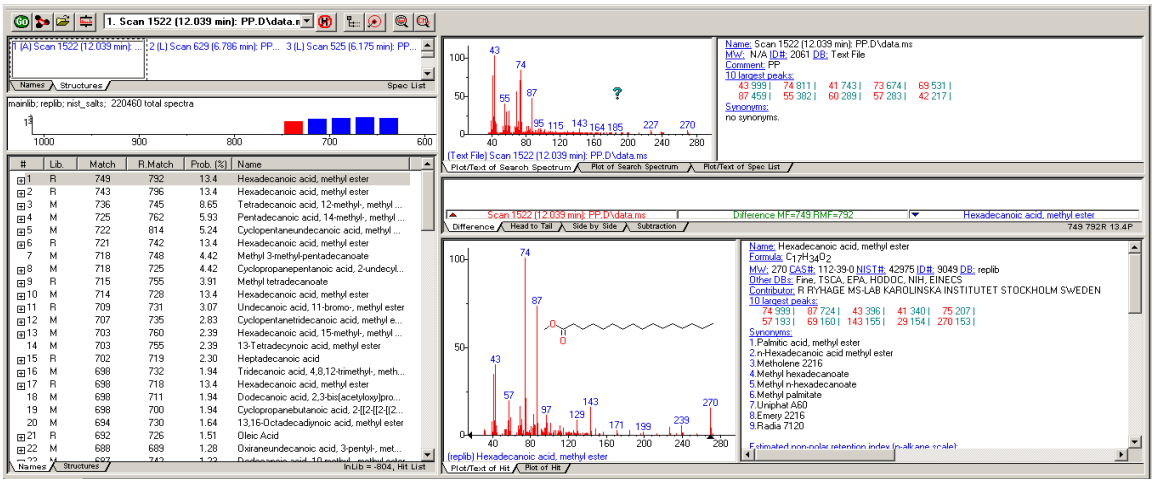

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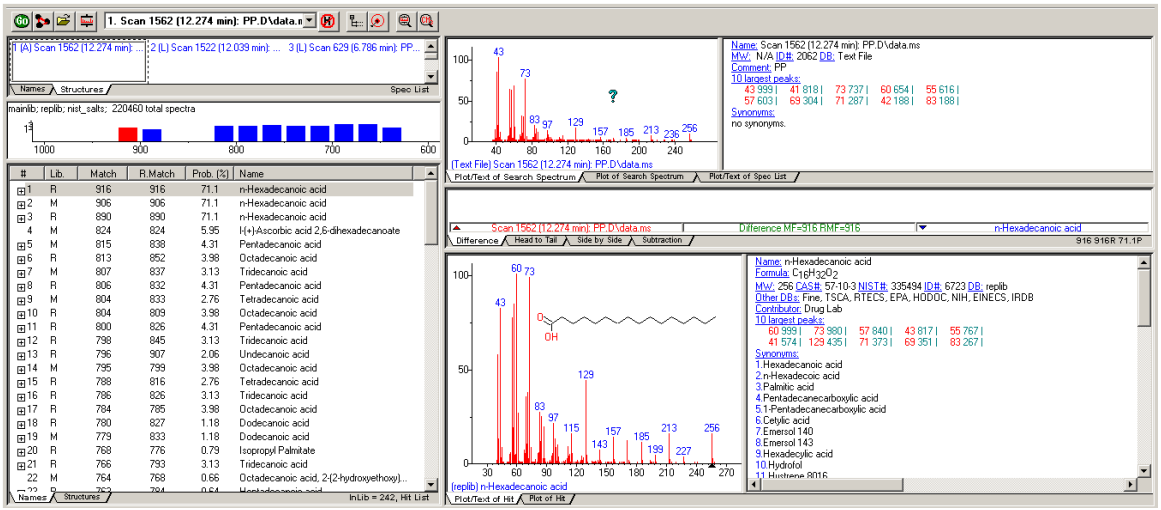

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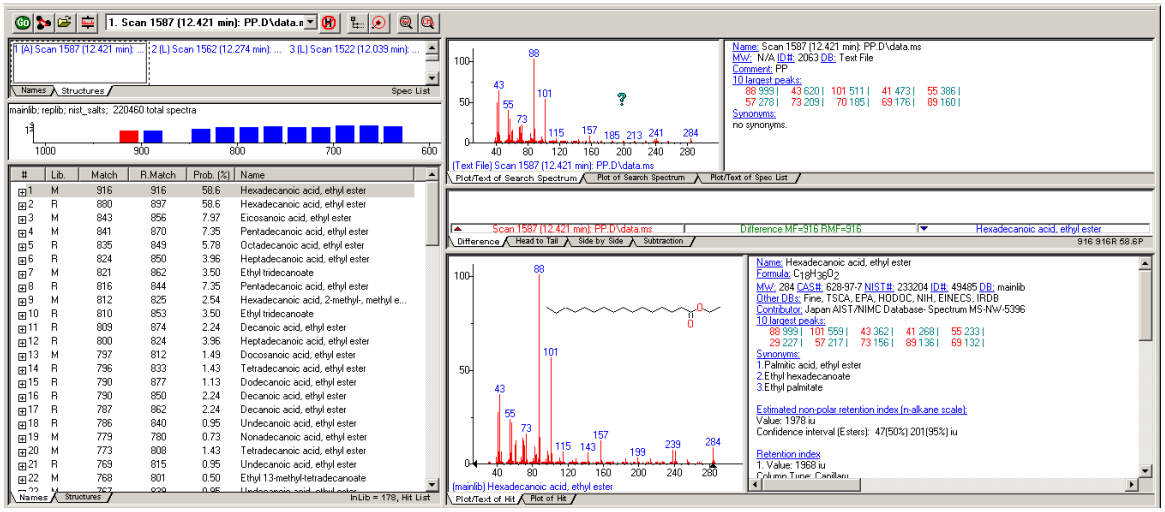

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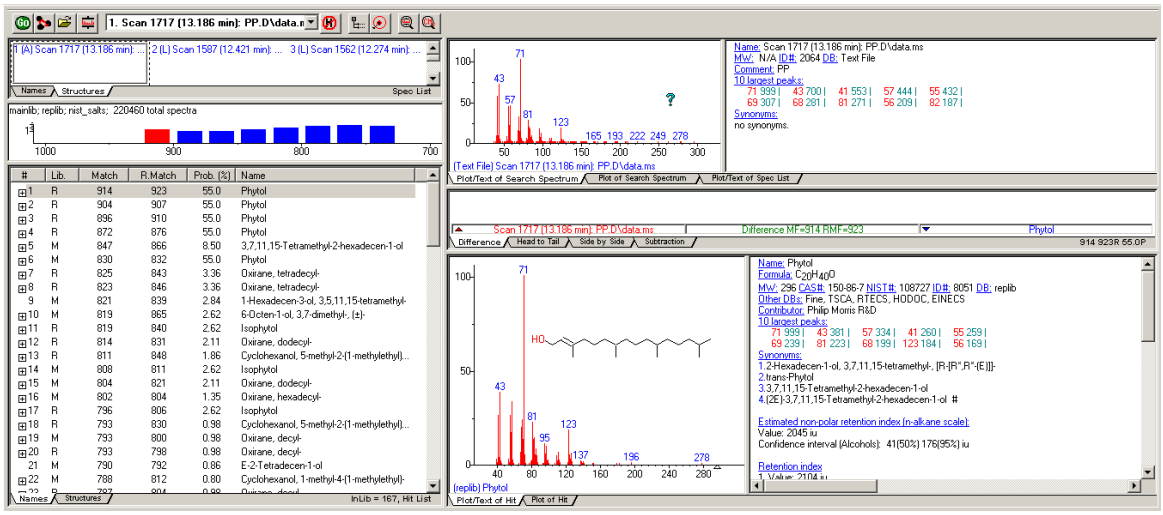

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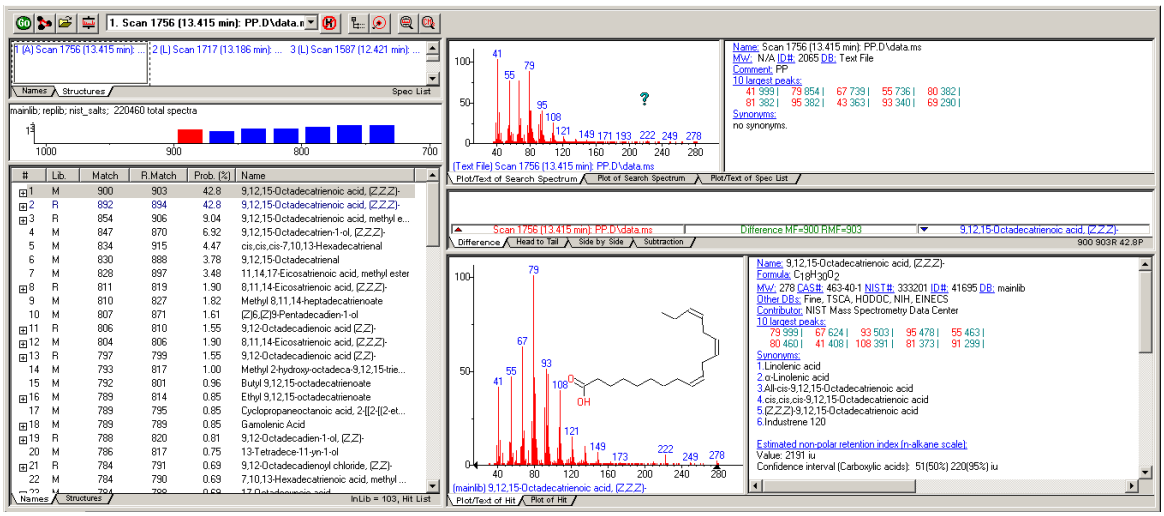

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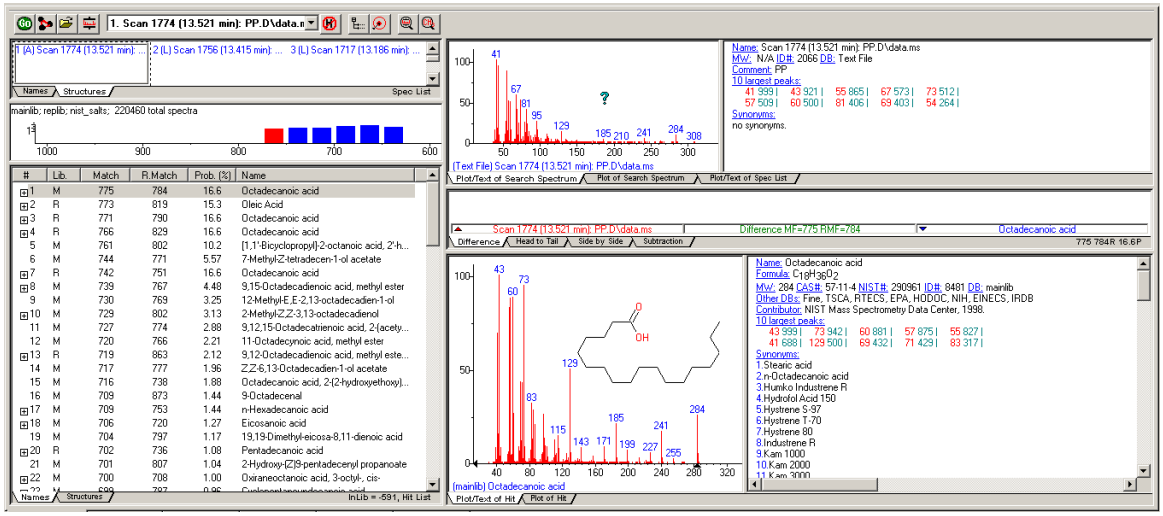

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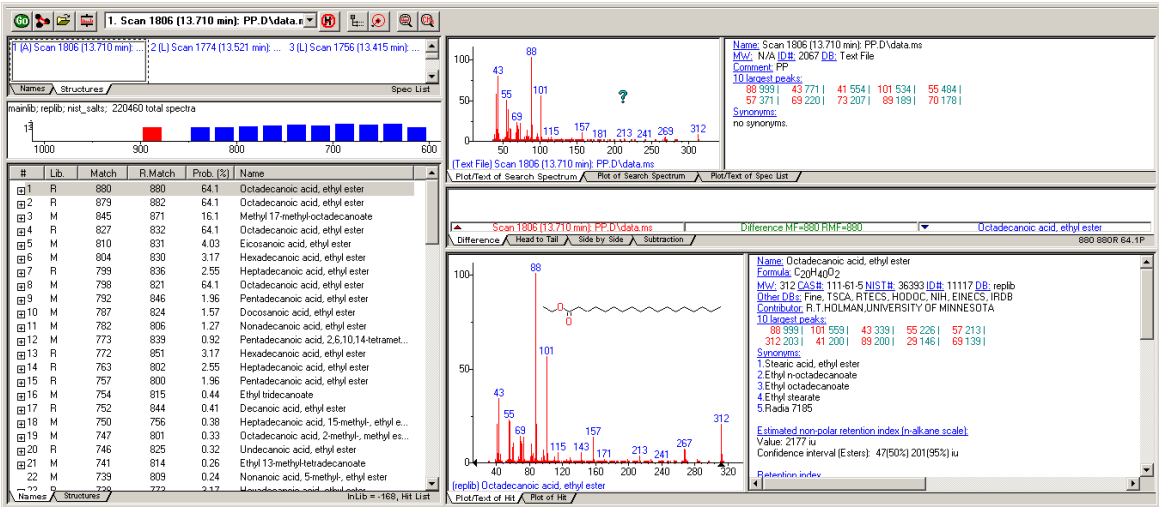

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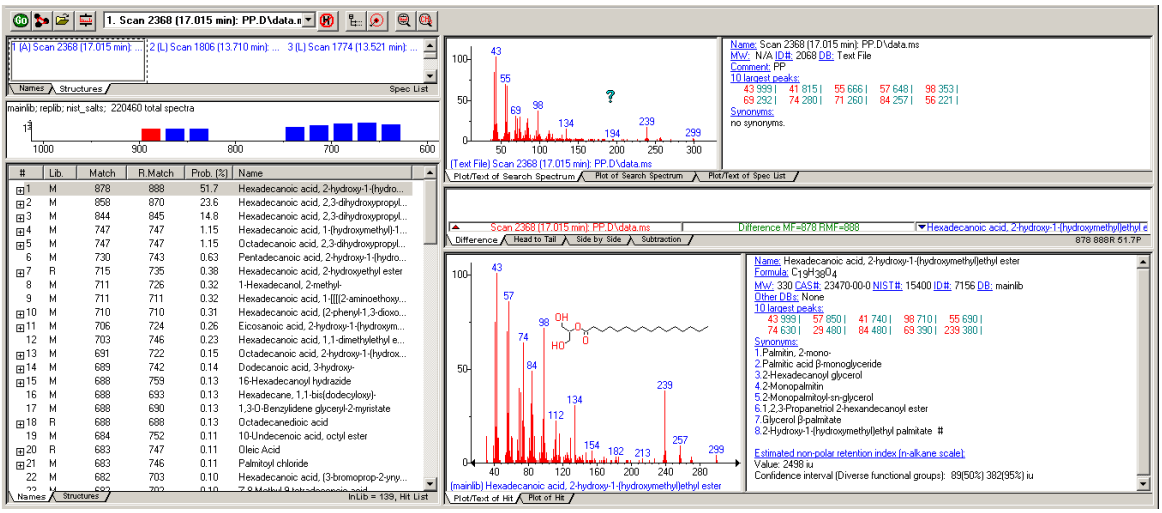

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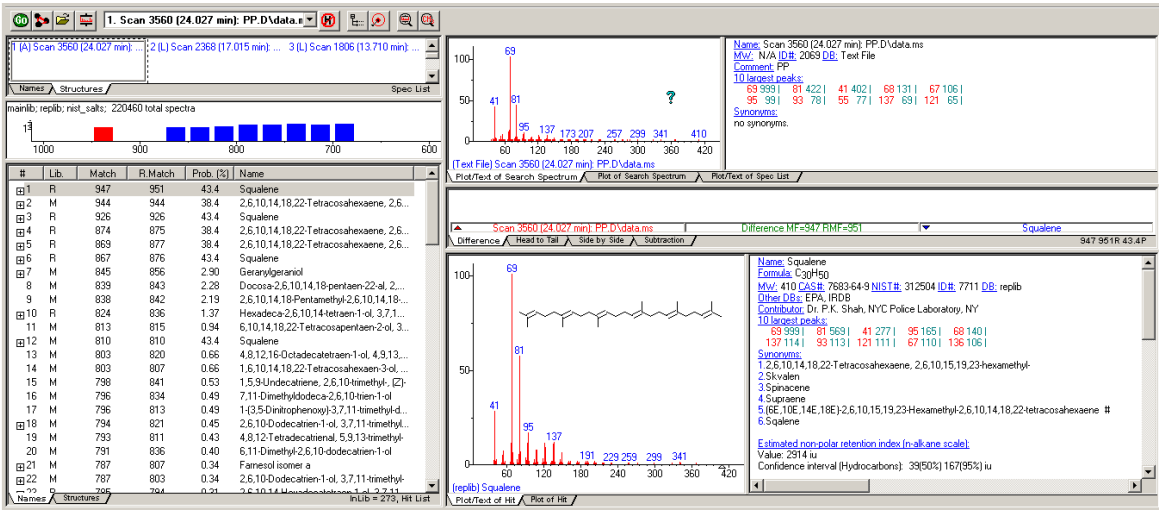

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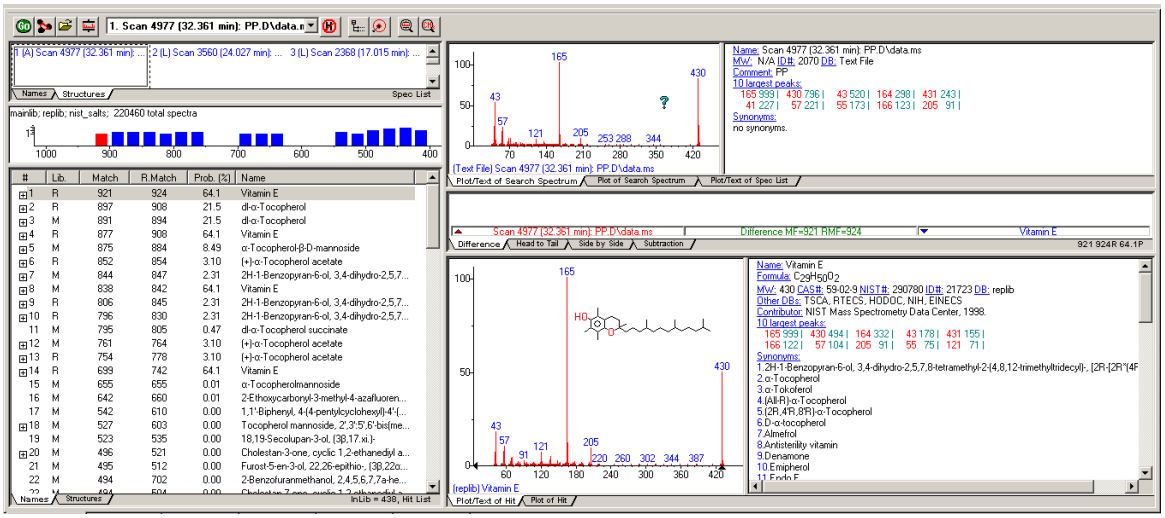

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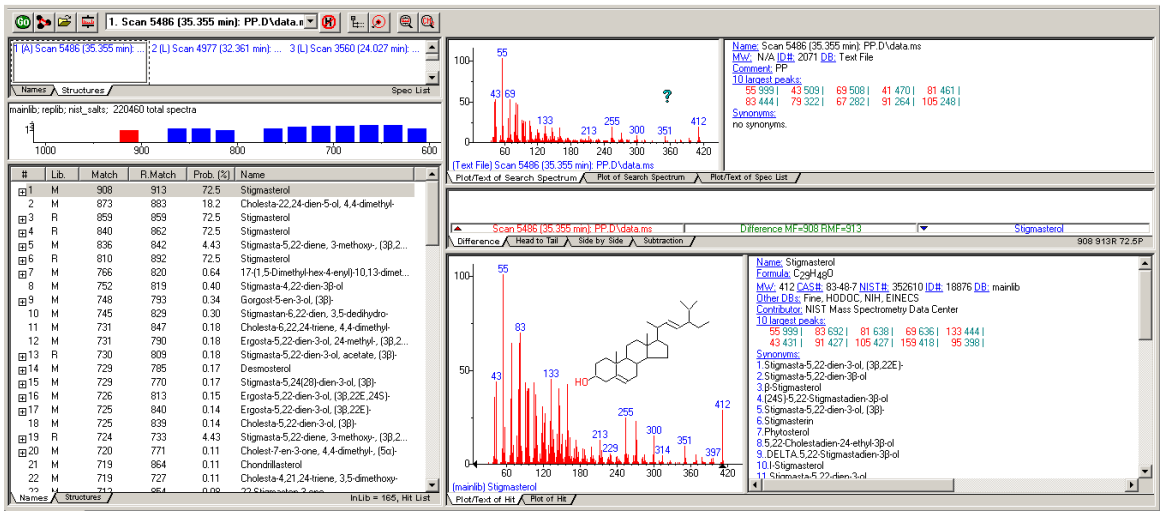

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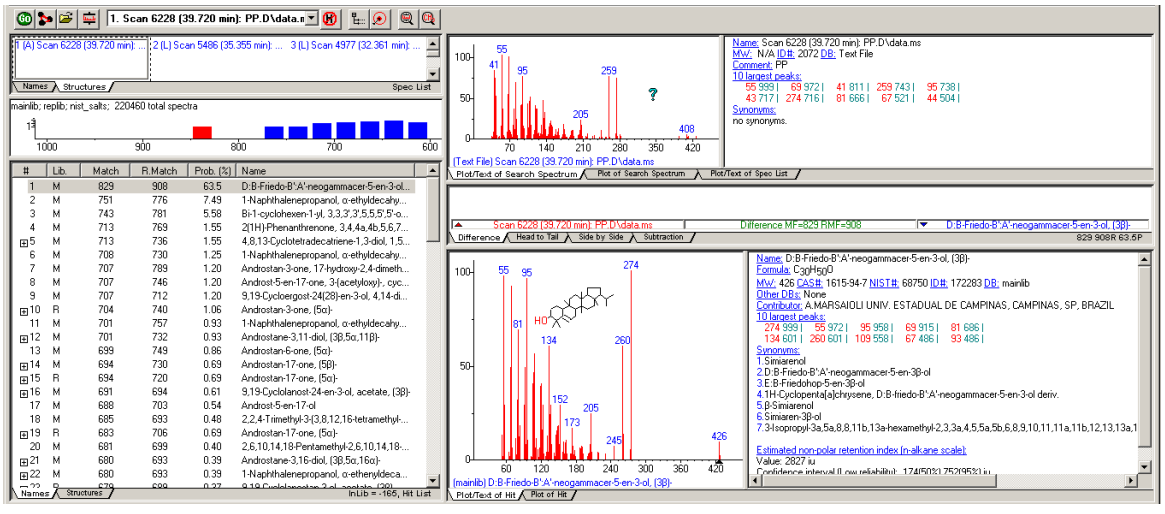

39.720

CONCLUSION

Findings from the GC-MS analysis, revealed the presence of active principles which are responsible for pharmacological activities inherent in the plant. These includes; antimicrobial, antifungal, antioxidant, anti-inflammatory, hypocholesterolemic, antiarthritic, antiandrogenic activities and many more. Also the pylorus ligation induced model confirmed that *Peuraria phaseoliodes* leaves possess antiulcer potentials, which may be related with antisecretory as well as cytoprotective activities of one or more of the identified phytochemicals. Similarly, the antiulcer potentials of the study plant could equally be linked with the antioxidant and anti-inflammatory effects as revealed by the GC-MS analysis. Thus promoting the usage of herbal medicine. Hence, GC-MS can be recommended among the first step towards understanding the nature of active principles.

Future research

Recommendation of more analytical tests are necessary because through them, more important components of the plant will be revealed thereby opening up many hidden potentials inherent in the plant.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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