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Comparative phytochemical investigation and biological evaluation of *Psoralea corylifolia*

Promod Kumar, Sandip Sen*, Manish Shakya and T. S. Easwari

IIMT College of Medical Sciences, O-Pocket, Ganga Nagar, Meerut

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ABSTRACT

P. corylifolia is a medicinally important herb belongs to family Fabaceae. The present investigation was carried out on seed and root parts for comparative phytochemical and biological screening like antimicrobial, analgesic, smooth muscle constriction and antidiabetic activity of different extracts. The extractions were carried out using solvents like petroleum ether, methanol and acetone. The yield value indicated methanol is a suitable solvent compared to acetone and petroleum ether. The proximate analysis showed root has more constituent compared to seed. From the analgesic and antidiabetic activity it was found that methanolic extracts of seeds and root were shown more potent activity, compare to standards. Whereas root extracts of acetone and petroleum ether showed moderate activity. The extracts of Psoralea corylifolia were shown smooth muscle constriction effect on isolated rat illium at dose dependent manner. From antimicrobial study it was reported that extracts of both seed and root were shown good results against gram positive bacterias and fungi. There was no promising effect was found in case of selected gram negative bacterias. The petroleum ether extract were not even shown any significant effect in case of fungal strains.

Key words: *P. corylifoila*, Analgesic, Antidiabetic, Muscle constriction, Antimicrobial.

INTRODUCTION

Medicinal plants would be the best source to obtain a variety of drugs [1]. Historically it was found that active constituents obtained from natural origin considered as valuable source for innumerable therapeutic agents [2]. World Health Organization estimates that up to 80 percent of people still used herbs as a source of medicines in traditional remedies [3]. *P. corylifolia* is a medicinally important belongs to family Fabaceae. It is an erect annual, 30-180 cm high plant and well recognized in Chinese and Indian folkloric medicine. The fruits of *P. corylifolia* consist of a sticky oily pericarp, a hard seed coat and kernel. The seeds are used in indigenous medicine as laxative, aphrodisiac, antihelminitic, diuretic and diaphoretic in febrile conditions. The seeds have been specially recommended in the treatment for leucoderma, leprosy, psoriasis and inflammatory diseases of the skin [4]. The seed extracts inhibits the growth of *Staphylococus citreus*, *S. aurues* and *S. albus* including strains resistant to penicillin's. The seeds posses psoralen exhibited antihlemintic activity against earth worms. The essential oil shows a selective activity against the skin *Streptococci* and used in the treatment of skin infections. The seeds are used locally in the preparation of certain types of medicated oils preparations. The root is useful in the caries of teeth [5]. Previous studies reported the presence of several constituents like, furanocoumarins [6], phenyl flavonoids, aromatic terpenoids and chromenes [7]. It is useful in inflammatory diseases [8], antitumor, antihyperglycemic, antidepressant, and antioxidant activities, asthma, cough, nephritis, antitumor, antibacterial, and antiviral properties. It is a good hair tonic and hence used in alopecia areata and hair loss [9]. They are also useful in fibrosarcoma and

leukemia. It has hepatoprotective properties, lumbago and tuberculosis. Essential oil is used as tonic and aphrodisiac, cytotoxic, antimutagenic, and antirepellant [10].

Hence in the present study, phytochemical screening and comparative biological evaluations like antimicrobial, analgesic, smooth muscle constriction, antidiabetic activity was carried out on seed and root part of *P. corylifoila.* The extractions were carried out using solvents system like petroleum ether, methanol and acetone.

EXPERIMENTAL SECTION

Plant Materials:
The seed and root parts of *P. corylifoila* were purchased from Khachedumal Mahaveer Prasad chemical and Indian herbal Drugs, Meerut. It was identified and authenticated by Dr. Rajsingh Saini, Dept. of Biotechnology, IIMT College of Medical Sciences, Meerut. The seed and root parts were air drying under sunshine and powered by grinding. Powdered drug were stored in air tight container at room temperature.

Preparation of *P. corylifoila* extract:
Solvent extracts of *P. corylifolia* were prepared by using seed and root as a plant material. Three different solvent systems like petroleum ether, methanol and acetone were used. 200g of powdered drug were used for each seed and root. The extraction was carried out successively in methanol, acetone and petroleum ether by continuous hot extraction process using Soxhlet apparatus at 65°C for 48 hours. The solvents like methanol acetone and petroleum ether were used according to polarity profile. After completion of extraction it was filtered and the solvent was removed by rotator evaporator. A dark brownish gummy mass of extracts obtained from both the seed and root were used for phytochemical study. The extract were designated as MSE, MRE (methanolic seed and root extract); ASE, ARE (Acetone seed and root extract); PESE, PERE (Petroleum ether seed and root extract) respectively.

Phytochemical screening of plant extracts [11, 12]**:**
A standard protocol is followed to detect the presence of various contents present in different parts (seed and root) of *P.corylifolia* as given in Table no-2.

Analytical parameters and proximate analysis:
Ash values:
Weighed accurately 2 g of air dried seed and root powder in tarred silica dish (silica crucible) and incinerate at a temperature not exceeding 450°C until free from carbon, cool and weighed. Collect the residue and on an ash less filter paper [12]. Incinerate the residue on filter paper until the ash is white or nearly so. Add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°C. Calculate the percentage of ash with reference to the air dried drug.

Ash % = Loss in weight (g) /w × 100
Where w = weight of the powder in grams.
Results are shown in Table no-3.

Acid insoluble ash:
The total ash obtained, was placed in silica crucible, added 25 ml of hydrochloric acid covered with a watch glass and boiled gently for 5 minute on a hot plate. Rinsed the watch glass with 5 ml of hot water and added these washing to the crucible. Collected the insoluble matter on ash less filter paper by filtration and rinsed that filter paper repeatedly with hot water until the filtrate was neutral. Transferred the filter paper containing the insoluble matter to the original crucible. Dried on a hot plate to a constant weight in the muffle furnace at 45°C. Removed the silica crucible from the muffle furnace, allowed for cooling in desiccators for 30 minutes and then weighed without delay.12 Calculate the content of acid insoluble ash in percentage as same above. Results are shown in Table no-3.

Extractive value (Cold maceration):
About 4g of air-dried material macerate with 100ml of solvent for 6 hours shaking frequently allow standing for 18 hours and filter. Transfer 25 ml of filtrate to tarred flat bottomed and evaporates to dryness. Drying was carried out at 105°C for 6 hrs [12]. Calculate the content of extractible matter in mg/g of air dried material. Results are shown in Table no-3.

Extractive value (%) = {(Wt. of dish + residue) (g) - Empty wt of the dish (g)} x 4 x100/wt
Where wt= weight of plant material taken in grams.

Biological Activity:
Animals:
Albino mice of either sex, weighed between 25-30 g were used for analgesic activity. Similarly wister rat of either sex weighed between 180-200 g were used for evaluation of anti diabetic and muscle contraction activity. Animals were maintained under standard environmental condition at temperature of 22 ± 2°C and 45-50 % relative humidity for 24 h each of dark and light cycle with proper diet. All the studies were done according to protocol approved by Institutional Animal Ethical Committee (IAEC) of IIMT College of Medical Sciences.

Acute toxicity study:
The acute oral toxicity study was performed according to OECD guideline no 423 for both albino mice and wister rats. The doses were fixed 100 mg/kg (p.o) to 2000 mg/kg (p.o) for both mice and rats contain 5 animal in each group. The mortality and general behavior were under observation for 14 days. The extracts were nontoxic in the dose of 250 mg/kg body weight [13].

Analgesic Activity:
Writhing Tests:
Mice of either sex with a weight between 20 and 25 g are used. 0.1 ml of a 0.6 % solution of acetic acid was injected intraperitoneally to mice [14]. The animals were consisted of 8 groups, 5 in each group. Group-1 was considered as control, Group-2 was used for aspirin (100 mg/kg) as standard and remaining groups were administered extracts MSE, MRE, ASE, ARE, PESE and PERE at the dose of 250 mg/kg bodyweight. The mice were placed individually into glass beakers for 5 mins to elapse. The mice were then observed for a period of 10 mins and the number of writhes was recorded for each animal. The time period with the greatest percent of inhibition is considered the peak time. A dose range was reserved for interesting compounds or those which inhibit writhing more than 70%. Compounds with less than 70% inhibition were considered to have minimal activity.

Tail immersion Test:
Young albino mice of either sex 25-30 gm were used for study [15]. The animals were consisted of 8 groups 5 in each group. Group-1 was considered as control, Group-2 was used for morphine sulfate (5 mg/kg) as standard and remaining groups were administered MSE, MRE, ASE, ARE, PESE and PERE at the dose of 250 mg/kg body weight. The extracts were administered 15 mins prior testing. The animals allowed adopting the environment before study. The tails were marked 5cm above and immersed in hot water of exactly 55°C. The tail withdrawal time was noted down before and after administration of drug in sec. The cut of time is 10 secs and 1-5 sec for treated and untreated animals respectively. If tail withdrawal time is more than 6 secs, indicates positive response.

Formalin test:
Formalin test was done by the method of Hunskaar *et al*. The animals were consisted of 9 groups 5 in each group. Group-1 was considered as control, Group-2 was used for morphine sulfate (5 mg/kg) as standard and Group-3 were used for aspirin (100 mg/kg) and remaining groups were used for extracts MSE, MRE, ASE, ARE, PESE and PERE. After 30 mins treatment of all extracts (15 min treatment for morphine), 20 µl of 2.5% formalin was injected subcutaneously in hind paw of rats [16]. The time spent in licking the injected paw in early phase (0–5 min) and late phase (15–30 mins) was recorded.

Oral Glucose Tolerance Test on Rat (OGTT):
There was 8 groups of animals which were administered normal saline solution of extracts at the dose of 250 mg/kg. Glucose solution was administered in the dose of 2 g/kg after 30min of administration of extracts. Blood sample was withdrawn from dorsal vein at interval of 60, 120 and 180 mins. Blood glucose level was estimated using blood glucose test strip with elegance glucometer [17] (Frankenbeng Germany) & GOD-POD kit (Acuurex,India).

Evaluation of antidiabetic activity:
Induction of diabetes:
Streptazocine (STZ) was used in the dose of 60 mg/kg to induce insulin dependent diabetes. STZ was injected into rats intraperitoneally [18]. After 48hrs of administration of STZ, the blood was collected by dorsal vein for

determination of blood glucose level. The rat with fasting glucose level in range of 275-300 mg/100ml were considered as diabetic and used for study.

Experimental protocol and dose schedule:
The total periods for conductance of study were 21 days. The rats were divided into several groups consisted of 5 animals in each group.

Group-1: Normal rats treated with vehicle alone saline 10 mL/kg by oral route
Group- 2: Diabetic control treated with STZ (60 mg/kg) dissolved in citrate buffer
Group-3: Diabetic rat treated with glebenclamide 10 mg/kg (Ranbaxy,India).
Group-4-9: Diabetic rat treated with extracts at 250 mg/kg body weight by oral route

On 1,7,14 and 21 days of study after 2 hours of oral administration of drugs blood glucose levels and body wt. were measured .Blood samples were withdrawn through dorsal vein. On 21 days whole blood was collected by cardiac puncture. Blood sample were centrifuged at 3000 rpm for 10 mins to obtained serum. Blood glucose levels were estimated by GOD-POD kit (Accurex.India).

Muscle constriction effect:
Preparation of dose:
The concentration of 10µg/ml of acetylcholine was used by dissolving in distilled water. For experimental purposes the dose for the plant extracts (MSE, MRE, ASE, ARE, PESE and PERE) was made by ethanol and water mixture (1:10) at the concentration of 0.01, 0.1 and 1 mg/ ml. Ethanol, at the same concentrations, had no effect on intestine contractility in the control experiments.

Tissue preparation:
Each rat was anesthetized by chloroform on the day of experiment. Then a 1.5–2 cm part was cut from the end of ileum and immediately put into a glass containers containing oxygenated tyrode solution in laboratory temperature. Then the sample was transferred into organ bath containing oxygenated tyrode solution at 37°C (pH 7.4) and was placed vertically between two stainless steel clasps [19]. Upper clasp was attached to isotonic transducer by thread and then from there to a physiograph device. 1 g weight was hung in front of transducer axel to provide first tension in tissue. Before starting the experiment, the Ileum was maintained in organ bath for 1 hour to be adjusted with new condition and the tyrode solution was changed every 15 minutes. After equilibrium period, a Dose Response Curve (DRC) for acetylcholine & extracts of *Psoralea corylifolia* seeds in variant molar concentrations was recorded. Increasing dose of acetylcholine (0.2- 0.8 ml), were added to the organ bath cumulatively to generate full concentration response curves. Then concentration response curves were obtained in the presence of the extract of *Psoralea corylifolia* in the organ bath. Percentage response can be calculated using following formula:

% Response = Minimum dose / Maximum dose x 100

Antimicrobial Activity:
Test microorganism and Medium:
For the determination of antimicrobial activity gram positive bacteria *Staphylococcus aureas* ATCC12600, *Bacillus subtilis* ATCC 11775, *Enterobacter cloacae* ATCC13047 and the fungi *Candida albicans* ATCC 90028, *Aspergillus niger* ATCC1027 were used. Bacterial strains were cultured overnight at 37ºC in LB-agar broth and fungal strains were cultured over night at 30ºC in cornmeal agar media. Test strains were suspended in nutrient agar to give final density of 5X105 cfu/ ml.

Screening for antimicrobial activity (Zone of inhibition assay):
Antimicrobial activities [20] of the extracts were determined by disc diffusion method. Sterilized 10 % nutrient agar (20ml) was poured into each sterile petridish after mixing the culture of micro-organism at the concentration of 150 µl and allowed to solidify the plate. Standard and test compounds were dissolved in DMSO to make a stock solution of 1000 µg/ml. From the stock solution, concentration of 200, 350, 500µg/ml made for amoxicillin, streptomycin, nystatin and extracts (MSE, MRE, ASE, ARE, PESE, PERE) respectively. Whattmann filter paper was sterilized and dipped in test compounds, standards and solvent control respectively. Discs were placed on agar plates and incubated at 37°C for 24hrs for bacterial and 30°C for 24 hrs for fungi and the zone of inhibition was measured in millimeter. Each study was performed in triplicate.

Statistical Analysis:
The results were shown as Mean ± SEM and comparison between standard and test compounds were made by one way ANOVA followed by Dunnetts test. Values of $p \leq 0.001$ were considered as significant.

RESULTS AND DISCUSSION

From the yield value it was found that methanol is a suitable solvent for the extraction process. The phytochemical investigation confirmed the presence of alkaloids, coumarins, fattyacids, lipids, aucubins, iriodoids in *P.corylifolia.* From analytical parameters and proximate analysis it was accounted that the roots part has more ash value compared to seed. The extractive value signifies that alcohol is a suitable solvent from extraction process

Table-1: Percentage yield Value of different extract

Extract code	Name of Extract	Percentage yield
MSE	Methanolic seed extract	15.9
MRE	Methanolic root extract	18.6
ASE	Acetone seed extract	13.2
ARE	Acetone root extract	16.3
PESE	Petroleum ether seed extract	14.1
PERE	Petroleum ether root extract	13.8

Table no.2: **Presence (+) and absence (-) of active constituent in *P.corylifolia* in different solvents during phytochemical screening**

Active Constituent	Solvent System					
	Methanol		Acetone		Petroleum Ether	
	Seed	Root	Seed	Root	Seed	Root
Alkaloids	-	+	-	-	+	-
Aucubins/Iriodoids	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+
Tannins	-	+	-	-	+	-
Anthocyanins/Anthocyanidins	-	-	-	-	-	-
Anthracene Glycosides	-	-	-	-	-	-
Carotenoids	+	-	+	+	+	-
Cynogenic Glycosides	-	-	-	-	-	-
Steroids	+	-	+	-	+	-
Emodins	-	-	-	-	-	-
Fattyacids/ Lipids	+	+	+	+	+	+
Flavonoids	-	+	-	-	-	-
Triterpenoids	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

Analytical parameters and proximate analysis:

Table No-3: Ash and Extractive Value of powder seed and root

Sr. No	Parameter (w/w)	Value (%)	
		Seed	Root
1	Total ash	7.4	8.4
2	Acid insoluble ash	1.89	2.89
3	Alcohol soluble extractives	13.8	19.8
4	Water soluble extractives	11.8	21.8

The analgesic activities of extracts were determined by the acetic acid-induced writhing tests (Table-4), tail immersion (Table no-5) and by the formalin-induced pain method (Table no-6). It is well established that thermal nociceptive tests are more sensitive to opioid μ-agonists and non-thermal tests to opioid κ-agonists [21]. Aspirin produces analgesia through inhibition of prostaglandin synthesis. The abdominal contraction response induced by acetic acid used for peripherally acting analgesics and such a response was thought to involve local peritoneal receptors. The tail immersion and tail flick tests caused a profound and dose related analgesia in the treated mice, which are behavioral methods that have been developed to study nociception in animals [22]. The data generated in the present study suggests that the involvement of both κ and μ opioid receptor are distinct in the analgesic activity of compounds.

Table- 4: Analgesic activity of extracts on mice: Writhing Tests

Dose (mg/kg)	Mean % inhibition ± SEM			
	Without drug	After 30 min	After 60 mins	After 90 mins
Control (0.2ml/kg)	-	-	-	-
Aspirin(100)	96.30±0.09	75.87±0.01	78.69±0.18***	94.19±0.18***
MSE	93.60±0.01	68.77±0.04	76.16±0.11***	93.16±0.11***
MRE	93.04±0.19	69.86±0.07	72.84±0.15**	82.24±0.15***
ASE	82.31±0.13	21.31±0.20	34.36±0.18	25.16±0.18*
ARE	87.13±0.09	58.22±0.18	67.14±0.22**	79.24±0.22**
PESE	85.57±0.11	39.15±0.13	41.13±0.16	38.18±0.15
PERE	82.14±0.07	38.02±0.05	49.95±0.12**	69.45±0.12**

****P<0.0001 considered as significant;**P<0.001 considered as moderately significant. Data were analyzed by student's t-test for n=6.*

Table-5: Analgesic activity of extracts on mice: Hot immersion

Dose (mg/kg)	Mean % inhibition ± SEM			
	Without drug	After 30 min	After 60 mins	After 90 mins
Control (0.2ml/kg)	-	-	-	-
Morphine(5)	96.30±0.09	75.17±0.01	78.69±0.18***	90.19±0.18***
MSE	83.60±0.02	57.17±0.03	76.16±0.11***	88.16±0.11***
MRE	83.04±0.01	55.86±0.05	62.04±0.15**	85.04±0.15***
ASE	82.31±0.12	28.31±0.02	34.06±0.18	25.16±0.18*
ARE	87.13±0.01	33.22±0.01	57.04±0.22***	69.04±0.22***
PESE	84.57±0.10	39.15±0.03	41.03±0.16	38.18±0.15**
PERE	83.14±0.06	46.02±0.05	42.05±0.12***	67.05±0.12***

After immersion in water at 55ºC

****P<0.0001 considered as significant;**P<0.001 considered as moderately significant. Data were analyzed by student's t-test for n=6.*

Table-6: Analgesic activity of extracts on Phase-1 and Phase-2 by formalin induced analgesia

Dose (mg/kg)	% of Time spent in licking paw (secs) ± SEM		
	Without drug	0-5 mins	15-30 mins
Control (0.2ml/kg)	-	-	-
Morphine(5)	82.01±0.14	85.27±0.01***	86.19±0.18***
Aspirin(100)	85.00±0.11	85.87±0.31***	89.69±0.18***
MSE	78.08±0.01	78.27±0.24***	77.66±0.11***
MRE	81.34±0.21	79.86±0.27***	76.84±0.15
ASE	85.61±0.11	47.30±0.21	48.26±0.18
ARE	82.63±0.21	68.22±0.18***	66.14±0.22**
PESE	83.20±0.31	49.15±0.13***	51.13±0.16**
PERE	87.63±0.21	67.02±0.05**	65.95±0.12**

****P<0.0001 considered as significant;**P<0.001 considered as moderately significant. Data were analyzed by student's t-test for n=6.*

The formalin-induced pain as an experimental model of analgesia is useful for elucidating mechanism of pain and analgesia. Drugs that act centrally, such as the narcotics inhibit both phases of formalin-induced pain, while peripherally acting drugs can only inhibit the late phase [23]. From the result it was found that root extracts were showing more potent analgesic activity compare seed extract. Where as compared to standards (aspirin and morphine sulphate) metha nolic extracts are more significant. From the result it was also found that methanolic extracts of seeds and root were shown more potent activity, where as root extracts of acetone and petroleum ether showed moderate activity.

The antidiabetic activities were determined by STZ induced methods. Streptozotocin selectively destroys the insulin secreting ß-cells, leaving less active cells and resulting in a diabetic state. STZ-induced diabetes is characterized by severe loss in body weight and this reduction is due to loss or degeneration of structural proteins, as the structural proteins are known a major contributor to body weight. The antidiabetic potentiality of the *Psoralea corylifolia* extract has been supported by the recovery in the activity of hexokinase and glucose-6-phosphate dehydrogenase. As these enzymes are insulin sensitive, so it may be postulated that the composite extract may protect these enzymes by recovery of plasma [23]. Blood glucose level in rats administered with 2g/kg glucose was significantly decreased by extracts with in 1 hour as compare to standard glibenclamide.

Treatment with extracts showed that there was significant fall of blood glucose level compared to standard glibanclamide on 21 day of study. Diabetes was also characterized by severe loss of body wt. On 21 day of

treatment it was found that there was significant gain of body wt. specially the rat treated with test compounds. From data analysis it was found that root extracts were showing more potent activity than seed extract compare to standards glibenclamide. From the result it was also established that methanolic extracts of seeds and root were shown more potent activity, where as root extracts of acetone and petroleum ether showed moderate activity. No significant effects were found in case of seed extract of acetone and petroleum ether.

Antidiabetic Activity of extracts by Streptazocine induced method

Table-7: Change in OGTT blood glucose level (mg/dl) by extracts

Dose (mg/kg)	Change of OGTT blood glucose level (mg/dl) by extracts in Mins ± SEM			
	0	60	120	180
Control	91.5±2.23	106.2±3.02	103±7	93.67±1.00
Standard	91.5±2.23	110.0±3.94	108±1.89	97.83±0.47
MSE	95±2.23	125.3±1.22	110.±1.28	99±0.96***
MRE	95±2.23	128 ±0.93	114±2.6	97.8±0.27***
ASE	89.25±2.23	138±0.94	121±2.81	118.17±0.70
ARE	91.17±1.30	141.3±0.91	130.5±2.43	96±0.85***
PESE	92.83±0.87	136.6±0.87	125.5±1.83	193.5±1.4
PERE	93.83±0.6	152.2±7.34	132±0.85	90.83±0.47***

****P<0.0001 considered as significant;**P<0.001 considered as moderately significant. Data were analyzed by student's t-test for n=6*

Table-8: Fasting blood glucose level (mg/dl) by extracts on different days of treatment

Dose (mg/kg)	Change in Fasting blood glucose level (mg/dl) by extracts on Number of days ± SEM			
	0	7	14	21
Control	91±1.00	115.7±3.9	114±6.8	100±2.47
Diabetic control	244.7±2.96	281.3±2.51	311±3.99	336±2.12
Standad	311.3±3.99	247.8±7.4	126.7±5.8	106.5±1.9
MSE	285.3±5.28	195±6.58	152±7.66	108.17±1.35***
MRE	293.5±4.35	191±6.79	150.5±7.55	105.5±1.76***
ASE	283.2±1.19	190±3.73	157±6.09	190.81±2.8
ARE	293.5±6.99	158.2±3.20	127.3±2.01	117.33±0.5**
PESE	285.2±4.79	149.8±2.89	129±1.88	198.33±0.76
PERE	301.7±3.07	152.5±1.2	132.2±1.65	127.83±0.87***

****P<0.0001 considered as significant;**P<0.001 considered as moderately significant. Data were analyzed by student's t-test for n=6*

Table-9: Change in Bodyweight (gm) of wisterrats by extracts on different days of treatment

Dose (mg/kg)	Change in Bodyweight (gm) of wister rats by extracts Number of days ± SEM			
	0	7	14	21
Control	160±0.6	169±0.5	166±0.8	163±0.2
Diabetic control	178.5±0.99	190±0.69	215±0.56	253±0.12
Standad	171±0.39	144.5±0.67	141±0.60	169±0.6
MSE	163.3±0.61	215.2±0.69	187±0.89	178±0.9***
MRE	174±0.49	133±0.57	127.8±0.27	167±0.61***
ASE	167.7±0.49	143±0.57	129.8±0.55	129.2±0.46
ARE	166±0.36	142±0.36	125±0.83	148±0.69**
PESE	167±0.41	131±0.5	127±0.56	123±0.26
PERE	168±0.51	141±0.6	137±0.4	152±0.3**

****P<0.0001 considered as significant;**P<0.001 considered as moderately significant. Data were analyzed by student's t-test for n=6*

Acetylcholine binds to muscarinic receptors on ileal smooth muscles causing the receptor-operated channel to open thus allowing sodium influx, which causes depolarization of the cell membrane. This depolarization opens voltage dependent calcium channels and calcium ions enter the cell to induce the release of calcium from sarcoplasmic reticulum [19]. The cytosolic calcium then binds to calmodulin and contraction is produced. Acetylcholine (10µg/ml) caused a concentration dependent contraction of the isolated rat illium, while the extract (0.01-1 mg/ml) produced a dose dependent spontaneous contraction of the ilium. Unlike analgesic and antidiabetic activity it was found that all the extracts of *Psoralea corylifolia* were shown smooth muscle constriction ettect on isolated rat illium at dose dependent manner.

Table-10: Effect of muscle constriction effect of *Psoralea corylifolia* extracts on rat ileum

Sr. No	Group	Dose (mg/ml	Dose (ml)			Response in Hight (cm)			% of response (Avg)		
1	Normal Saline	5	0.2	0.4	0.8	-	-	-	-	-	-
2	Ach	10 µg/ml	0.2	0.4	0.8	1.5	3	6	25	50	100
3	MSE	0.01	0.2	0.4	0.8	0.7	1.2	2	11.67	60	100
4		0.1	0.2	0.4	0.8	1.1	1.8	2.2	18.33	81.82	100
5		1	0.2	0.4	0.8	1.5	2.1	3	25	70	100
6	MRE	0.01	0.2	0.4	0.8	1.2	2	2.5	20	80	100
7		0.1	0.2	0.4	0.8	1.5	2.3	3.3	25	69.7	100
8		1	0.2	0.4	0.8	2	2.8	3.9	33.33	71.79	100
9	ASE	0.01	0.2	0.4	0.8	0	0.8	1.2	0	66.67	100
10		0.1	0.2	0.4	0.8	1.1	1.8	2.6	18.33	69.23	100
11		1	0.2	0.4	0.8	1.4	2.1	2.9	23.33	72.41	100
12	ARE	0.01	0.2	0.4	0.8	0.5	1.3	2.1	8.33	61.9	100
13		0.1	0.2	0.4	0.8	1.5	2.6	3.2	25	81.25	100
14		1	0.2	0.4	0.8	1.9	2.9	3.8	31.67	76.32	100
15	PESE	0.01	0.2	0.4	0.8	0.5	1.3	1.9	8.33	68.42	100
16		0.1	0.2	0.4	0.8	1.5	2.1	2.9	25	72.41	100
17		1	0.2	0.4	0.8	1.9	2.5	3.2	31.67	78.13	100
18	PERE	0.01	0.2	0.4	0.8	0.6	1.8	2.7	10	66.67	100
19		0.1	0.2	0.4	0.8	1.5	2.6	3.1	25	83.87	100
20		1	0.2	0.4	0.8	1.8	2.9	3.7	30	78.38	100

The antimicrobial activity was determined by paper disc diffusion technique against different strains of gram positive and gram negative bacteria and fungi at the dose of 200 µg, 350 µg, and 500 µg/ml shown in the Table-11 and 12. From the results it was found that the methanolic extracts of both seed and root were shown good results against gram positive bacterias and fungi. There was no promising effect was found in case of selected gram negative bacterias. On comperative analysis between different extracts it was focused that methanolic extracts was more potent compared to acetone and petroleum ether extract. Even it was depicted that petroleum ether extract were not even shown any significant effect in case of fungal strains.

Table-11: Invitro Antibacterial study by disc diffusion technique of different extracts

Extracts	Gram Positive bacteria						Gram negative bacteria					
	Staph aureus			*Bacillus substilis*			*Escherichia coli*			*Enterobacter cloacae*		
	Zone of inhibitions(mm) / Concentration (µg/ml)											
	200	350	500	200	350	500	200	350	500	200	350	500
MSE	13	15	16	11	13	15	5	7	9	x	x	x
MRE	15	18	20	18	19	22	6	7	10	x	x	x
ASE	14	15	17	17	18.1	19	x	x	x	x	x	x
ARE	11	13	18	14	15	17	x	x	x	x	x	x
PESE	13	14	15	11	13	17	x	x	x	x	x	x
PERE	10	14	18	14	13	18	x	x	x	x	x	x
Amoxycillin	10	12	14.1	12	14	17.1	13	18	22.1	12	14	17.1
Streptomycin	11	13	16	11	13	16	19	21	22	11	13	16

Table-12: Invitro Antifungal study by disc diffusion technique of different extracts

Extracts	Fungi					
	Aspergillus niger			***Candida albicans***		
	Zone of inhibitions(mm) / Concentration (µg/ml)					
	200	350	500	200	350	500
MSE	10	11	15	10	14	16
MRE	12	13	18.3	15	17	19
ASE	13	14	18	16	18	19
ARE	15	17	18.3	15	17	19
PESE	x	x	x	x	x	x
PERE	x	x	x	x	x	x
Nystatin	18	19	20	16	18	20

CONCLUSION

Methanol is a suitable solvent compared to acetone and petroleum ether. The proximate analysis indicated root has more constituent compared to seed. The biological evaluation like analgesic, anti-diabetic, invitro smooth muscle constriction and antimicrobial activity was determined. From the result it was found that methanolic extracts of seeds and root were shown more potent activity, compare to standards. Whereas root extracts of acetone and petroleum ether showed moderate activity. The extracts of *Psoralea corylifolia* were shown dose dependent spontaneous smooth muscle constriction ettect on isolated rat illium. The antimicrobial activity showed that the extracts of both seed and root were shown good results against gram positive bacterias and fungi. There was no promising effect was found in case of selected gram negative bacterias. The petroleum ether extract were not even shown any significant effect in case of fungal strains.

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