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ISOLATION OF PRELIMINARY PHYTOCONSTITUENTS AND ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY OF *CALOTROPIS GIGANTEA* LINN. LEAVES EXTRACTS

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ABSTRACT

The present studies that Isolation of preliminary phytoconstituents and anti-inflammatory and antipyretic activity of *Calotropis gigantea* Linn. Leaves Extracts. Therapeutic use of plants for the treatment of human illnesses dates back over man millennia. Evidence of their effectiveness in the diagnosis, cure and prevention of disease states exists in every culture throughout the world. Today "traditional medicine," characterized by the use of herbs and other natural products still remains a regular component of health care in countries such as China, Japan, India, South America and Egypt. The search for anti-inflammatory and analgesic agent in modern was marked by the introduction of salicin for the treatment of inflammatory swellings due to rheumatic fever and rheumatoid arthritis. The ethanol extract and distilled water extract showed good significant reduction in paw oedema as compared to control group, where as Petroleum ether (60-80^{o}C) extract, Chloroform extract, Ethyl acetate, n-Butanol has showed comparatively less significant reduction in paw oedema volume. The chloroform and n-butanol extract showed good significant reduction in rectal temperature as compare to control group, where as pet. ether, ethyl acetate, ethanol and distilled water extracts showed less significant reduction in rectal temperature. Hence, to put into the active principle of *Calotropis gigantea* linn like glycoside, sterols, carbohydrate, flavonoids, terpenoide may be responsible for anti-inflammatory and antipyretic activity.

INTRODUCTION: *Calotropis gigantea* Linn. (Asclepiadaeceae) found in dry waste places. Roots are externally whitish grey in colour. Leaves freshly, obovate, apex acute, rarely rounded, base cordate, 6-20 cm long and 3-8 cm wide. The stem is woody with yellowish white bark, young stem and branches covered with soft, loosely apprised, whitish, waxy or sometime powdery pubescence. Flowers are Lilac, pale rose or purple, rarely light greenish – yellow or white, inodorous [1].

Calotropis gigantea Linn. consist of cardiac glycosides, calotropin, calotoxin, syriogenin, proceroside, sterols, calctin. calotrposideA, calotroposide, calotropin D1 and D2, procerosterol, taraxsterol [2].

Therapeutically uses as Anti-cancer, intermittent fever, paralysed part of body painful joints, swelling, heals wounds (Leaves). The ethanolic (50%) extract of root exhibited anticancer activity (Roots). The latex is applied on wounds for quick relief also used as Antispasmodic, antiasthmatic, externally used for piles,

boils, Ulcers, Scabies, eczema, leprosy. It is having promising anti-inflammatory activity (Latex). It used as Asthma, cough, cold, catarrh, digestive, Stomachic, tonic (Flower) [3].

Herbs and plants are valuable not only for their active ingredients but also for their minerals, vitamins, volatile oils, glycosides, alkaloids, acids, alcohols, and esters and these component come from all parts of the plant including leaves, flowers, stem, berries, seeds, fruit, bark and roots [4].

Inflammation is a dynamic pathological process consisting of a series of interdependent changes. It involves a series of events that can be elicited by numerous stimuli like infectious agents, ischemia, antigen-antibody interactions and thermal or physical injury. The common clinical signs include erythema, edema, tenderness and pain [5].

Fever is an elevation of body temperature above the normal circadian range as the result of a change in the thermoregulatory center located in the anterior hypothalamus. A normal body temperature is ordinary maintained, despite environmental variations, through the ability of the thermoregulatory center to balance heat production by the tissues with heat dissipation [6].

MATERIAL & METHODS:

Procurement of Drug : The leaves of *Calotropis gigantea* linn. were collected from local area of Toranmaal District Nandurbar.

Drying & Size Reduction: *Calotropis gigantea* linn. Were shade dried under normal environmental conditions and then subjected for size reduction to coarse powder [7].

Extraction: The dried material was reduced to coarse powder in a mechanical grinder to obtain about powder of desired particle size. About 1 kg of powdered material was subjected to successive extraction with petroleum ether (60-80^{o}C), chloroform, ethyl acetate, n-butanol, ethanol and Distilled water. The extraction was continued until the solvent in the thimble becomes clear indicating the completion of the extraction. After each extraction, the solvent was distilled off and the extract was concentrated at low temperature [8].

The percentage yield of petroleum ether (60-80^{0}C), chloroform, ethyl acetate, n-butanol and ethanol, distilled water extract was recorded as under the show in **Table 1**.

TABLE 1: PERCENTAGE YIELD OF *CALOTROPIS GIANTEA* LINN. LEAVES EXTRACTS

Extract	Weight of residue	% Yield
Petroleum-ether	35 gm	5%
Chloroform	10 gm	1.5%
Ethyl acetate	8 gm	1.27%
n-Butanol	7 gm	1.12%
Ethanol	15 gm	2.45%
Distilled Water	66 gm	11 %

Preliminary Phytochemical Investigation of Extract: The Result of preliminary phytochemical investigation of extract shown in **Table 2**.

Identification of Active Principle by TLC: The Chloroform, n-butanol, ethanol and distilled water extracts and were subjected to thin layer chromatography.

The details of TLC were as follows (for sterols)

Adsorbent : Silica gel G Activated.

Plate size : 20 cm x 8 cm.

Solvent : Chloroform: methanol [85:15]

Spraying reagent : Sulphuric acid and anisaldehyde.

Plate activation time : At 110^{o}C for 10 min.

The spot Visualized as pink color.

The Ethanol and n-butanol, chloroform extracts revealed the presence of sterols. The R_f values of each shown in **Table 3**.

The details of TLC were as follows (for flavonoids);

Adsorbent : Silica gel G Activated.
Plate size : 20 cm x 8 cm.
Solvent : Butanol : Acetic acid : water (5:1:4)
Spraying reagent : Chromatogram fumed with NH_3 and observed under UV.
Plate activation time : At 110^{o}C for 10 min [9].

The distilled water extract and ethanol extracts revealed the presence of flavonoids. The R_f values of each shown in **Table 4**.

TABLE 2: PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF *CALOTROPIS GIGANTEA* LINN. LEAVES EXTRACTS

Test	Petroleum ether extract	Chloroform extract	Ethyl Acetate extract	n-butanol extract	Ethanol extract	Distilled water extract
Test for Sterols						
a) test solution + Conc. H_2SO_4	-	+	+	+	+	+
b) Salkowski's Test	-	+	+	+	+	+
c) Test solution + Sulphur	-	+	+	+	+	+
d) Liebemann Burchardt's Test	-	+	+	+	+	+
Test for Glycosides						
a) Baljet's Test	-	+	-	-	-	+
b) Keller-Kiliani Test	-	+	-	-	-	+
c) Raymond's Test	-	+	-	-	-	+
d) Bromine Water Test	-	+	-	-	-	+
e) Legal's Test	-	+	-	-	-	+
Test for Carbohydrates						
a) Molisch's Test	-	+	+	+	-	+
b) fehlings Test	-	+	+	+	-	+
c) Barfoed's Test	-	+	+	+	-	+
d) Benedict's Test	-	+	+	+	-	+
Tests for Flavonoids						
a) Ferric Chloride Test	-	-	-	-	+	+
b) Shinoda Test	-	-	-	-	+	+
c) Zn-HCL reduction Test	-	-	-	-	+	+
d) Alkaline reagent Test	-	-	-	-	+	+
e) Lead acetate Test	-	-	-	-	+	+

TABLE 3: R_F VALUES OF ETHANOL AND N-BUTANOL, CHLOROFORM EXTRACTS OF *CALOTROPIS GIANTEA* LEAVES

Extracts	R_f Values	Colure
Ethanol	0.96	Pink
	0.54	Blue
n-butanol	0.93	Pink
	0.50	Blue
Chloroform	0.91	Pink
	0.55	Blue

TABLE 4: R_F VALUES OF ETHANOL AND DISTILLED WATER EXTRACTS OF *CALOTROPIS GIANTEA* LEAVES

Extracts	R_f Values	Colure
Ethanol	0.89	Green
	0.55	Blue
Distilled water	0.90	Green
	0.57	Blue

MATERIALS AND METHODS:

(Antiinflammatory And Antipyretic Activity):

Animals Selection: Female mice weighing between 20-25 gm. were used for acute toxicity study of various extracts. The animals were fasted overnight prior to the acute experimental procedures.

Albino rats, wistar strain, of weighing 100-150 gm were used for acute model. Rats were kept in polypropylene cages and feed on standard laboratory diet. The animals were exposed to 12 hours of darkness and light each. The bedding material of cages was changed everyday. Rats were divided into fourteen groups of six each.

Materials used:

A) Extracts used:

1. Petroleum ether extract
2. Chloroform extract
3. Ethyl acetate extract
4. n-Butanol extract
5. Ethanol extract
6. Distilled Water extract

B) Paracetamol
C) Carrageenan (Sigma Chemicals, USA)
D) Plethysmometer (Mercury Displacement method)

Acute Toxicity Study: Acute toxicity study was carried out according to OECD guidelines (Organization for economic co-operation and development)[10]. The ethical clearance was obtained by the institutional Animal Ethics Committee (Registration number 652/02/a/CPCSEA) before the experiment.

Anti-inflammatory Activity:

Carrageenan induced Rat Paw Oedema: Rats were divided into eight groups of six each. They were starved overnight with water prior to the day of experiment.

Group I : Served as control (Inflammation induced)
Group II: Standard group Paracetamol 200 mg/kg b.w.
Group III: Petroleum ether extract 200 mg/kg
Group IV: Chloroform extract 200 mg/kg
Group V : Ethyl acetate extract 200 mg/kg
Group VI : n-Butanol extract 200 mg/kg
Group VII : Ethanol extract 200 mg/kg
Group VIII : Distilled Water extract 200 mg/kg

Thirty minutes after drug or test compound administration, 0.1 mL. of 1 % carrageenan in distilled water was injected into the subplantar region of right hind paws of all groups. A mark was put on the leg at the malleolus to facilitate uniform dipping at subsequent readings. The paw oedema volume was measured with the help of plethysmograph by mercury displacement method, at zero hour. (Immediately after injecting carrageenan). The same procedure was repeated at 30 mins. 1, 2, 3 hours [10]. The difference between 1 hours and subsequent hours reading was taken as actual oedema volume. The percentage inhibition of paw oedema in the various treated groups was then calculated by using the formula;

$$\text{Percentage inhibition} = (1 - Vt/Vc) \times 100$$

Where Vt = is the oedema volume in the drug treated group. Vc = is the oedema volume in the control group [11].

Antipyretic Activity:

Yeast induced Hyperpyrexia Method: A 15% suspension of Brewer's yeast in 0.9% saline was prepared eight groups of 6 albino rats of either sex with body weight of 150-200 gm was used. By insertion of a thermocouple to a depth of 2 cm into the rectum the initial rectal temperature were recorded. The animals were fevered by injection of 10 mg/kg of brewer's yeast suspension subcutaneously in the back below the hope of the neck. The sight of injection was massaged in order to spread the suspension beneath the skin.

The room temperature was kept at $22^{o}C \pm 2^{o}C$. Immediately after yeast administration, food was withdrawn 18 hour. Post challenge, the rise in rectal temperature the measurement was repeated after 30 minute. Only animal with a body temperature of at least $38^{o}C$ are taken into the test. The animals react the test compound or standard drug by oral administration. Rectal temperature was recorded again 30, 60, 120, and 180 minute. post dosing [12].

RESULTS AND DISCUSSION:

Preliminary Phytochemical Investigation: Results of qualitative chemical investigation of leaves of Calotropis gigantea linn have indicated the presence of following active principles for various extracts;

Petroleum ether extract:		Mucilage and Fats
Chloroform extract	:	Glycosides, Sterols
Ethyl acetate extract	:	Sterol, Carbohydrates
n-Butanol extract	:	Carbohydrates, Sterols
Ethanol extract	:	Sterol, Carbohydrates, Flavonoids
Distilled water extract	:	Carbohydrates, Flavonoids, Sterols

Identification of Active Principles by TLC: The Presence of sterols in ethanol, chloroform and n-butanol extract was identified by TLC profile. The R_f value of the same was found to be as 0.96, 0.54, 0.91, 0.55, 0.93, 0.50. The Colour of the spot was pink and blue. The presence of flavonoids in ethanol and distilled water extract was identified by TLC profile. The R_f values were found to be 0.89, 0.55, 0.90, 0.57. The Colour of the spot was green and blue.

Acute Toxicity Study: Acute toxicity study was carried out according to OEDC (Organisation of Economic Cooperation and Development) guidelines in albino mice. The acute toxicity study of various extracts of *Calotropis gigantea* Linn leaves was showed signs of toxicity like tremour, convulsion and deep breathing at 2000 mg/kg body weight $1/10^{th}$ of the same dose for all these extract were taken as therapeutic dose i.e. 200 mg/kg. body weight

Anti-inflammatory study: The carrageenan- induced rat paw oedema indicated that, ethanol extract and distilled water has shown good significant ($p<0.001$) reduction in paw oedema to the extent of 8.30%, 12.7% at 200 mg/kg concentration, respectively from 1^{st} to 3^{rd} hour when compared to control group However, n-butanol extract, chloroform extract, ethyl acetate extract, petroleum ether ($60\text{-}80^{o}C$) extract, has reduced the paw oedema to the extent of 3.97%, 4.73%, 3.97%, 1.59% respectively at 200 mg/kg

concentration when compared with control group. Whereas Paraectamol also significantly (P<0.001) reduced paw oedema form 1st to 3rd hr when compared to control group (**Table 5**). It appears from the study that distilled water and ethanol extract of treated group showed good significant anti-inflammatory activity as compared to standard group, where as petroleum ether extract, chloroform extract, ethyl acetate extract and n-butanol treated group showed anti-inflammatory activity. The details of the result are indicated in Table 4 and **Figure 1**.

Anti-pyretic Study: The Yeast induced Hyperpyrexia method indicates that n-butanol and Chloroform extract has good significant reduction in rectal temperature to the extent of 37.30°C at 180 min. when compared to control group. The pet ether, ethyl acetate, ethanol and Distilled water have a significant reduction in rectal temperature. The details of the result are indicated in **Table 6** and **Figure 2**.

TABLE 5: PERCENTAGE INHIBITION OF CARRAGEENAN INDUCED RAT PAW OEDEMA METHOD BY USING *CALOTRHOPIS GIGANTEA* LEAVES EXTRACTS

Group	Test Material (dose)	Mean increase in paw volume and % inhibition		
		1 hr.	2 hr.	3 hr.
1.	Control	1.26 ± 0.152	1.27± 0.200	1.29 ± 0.116
2.	Standard (Paracetamol) 200mg/kg	1.08 ± 0.158 (14.29%)	1.11 ± 0.178 (12.6%)	1.2 ± 0.163 (6.98%)
3.	Petroleum ether extract (200mg/kg)	1.24 ± 0.879 (1.59%)	1.26 ± 0.110 (0.79%)	1.28 ± 0.486 (0.68%)
4.	Chloroform extract (200mg/kg)	1.24 ± 0.361 (2.37%)	1.21 ± 0.254 (4.73%)	1.27 ± 0.285 (1.56%)
5.	Ethyl acetate extract (200mg/kg)	1.21 ± 0.0740 (3.97%)	1.22 ± 0.081 (3.94%)	1.24 ± 0.223 (3.13%)
6.	n-Butanol extract (200mg/kg)	1.21 ± 0.191 (3.97%)	1.23 ± 0.204 (3.15%)	1.25 ± 0.159 (3.11%)
7.	Ethanol extract (200mg/kg)	1.23 ± 0.285 (8.30%)	1.19 ± 0.158 (5.56%)	1.22 ± 0.328 (5.43%)
8.	Distilled Water extract (200mg/kg)	1.10 ± 0.212 (12.7%)	1.2 ± 0.200 (5.52%)	1.21 ± 0.292 (6.21%)

The significance relative to respective control value: P < 0.001: N=6 (N: indicate No. of animals used in each group.)

TABLE 6: ANTIPYRETIC ACTIVITY OF VARIOUS EXTRACTS OF *CALOTROPIS GIGANTEA* AGAINT BREWER'S YEAST INDUCED PYREXIA IN ALBINO RATS

Group	Rectal Temp °C		Duration of Time after administration				
	Initial	18 hour after Yeast injection	30 Min	60 Min	90 Min	120 Min	180 min
Control	38.30 ± 0.03162	39.35 ± 0.0255	39.30 ± 0.0070	39.23 ±·0.0089	39.65 ± 0.0452	39.5 ± 0.0070	39.00 ± 0.004
Paracetamol	38.30 ± 0.0316	39.35 ± 0.0255	38.32 ± 0.0461	38.09 ± 0.0455	37.65 ± 0.0541	37.55 ± 0.0282	37.44 ± 0.008
Pet. ether	38.05 ± 0.0167	39.44 ± 0.0481	38.43 ± 0.0219	38.37 ± 0.0212	38.33 ± 0.0083	38.08 ± 0.0122	38.65 ± 0.008
Chloroform	38.28 ± 0.0141	39.48 ± 0.0054	38.92 ± 0.0122	38.00 ± 0.0083	37.92 ± 0.020	37.62 ± 0.0167	37.35 ± 0.007
Ethyl acetate	38.26 ± 0.0148	39.44 ± 0.0109	38.42 ± 0.0212	38.35 ± 0.0114	38.32 ± 0.0151	38.20 ± 0.0109	38.10 ± 0.007
n-butanol	38.25 ± 0.0122	39.41 ± 0.0167	39.01 ± 0.0260	38.08 ± 0.0207	38.62 ± 0.0568	38.12 ± 0.0328	37.65 ± 0.009
Ethanol	38.28 ± 0.0187	39.40 ± 0.0158	38.60 ± 0.0158	38.08 ± 0.0327	38.15 ± 0.0182	38.00 ± 0.0313	37.72 ± 0.018
Distilled Water	38.28 ± 0.0122	39.37 ± 0.0216	38.50 ± 0.0230	38.38 ± 0.0427	38.2 ± 0.0568	38.15 ± 0.0260	38.10 ± 0.032

The each value is a mean ± standard error for group of six animal (n = 6)

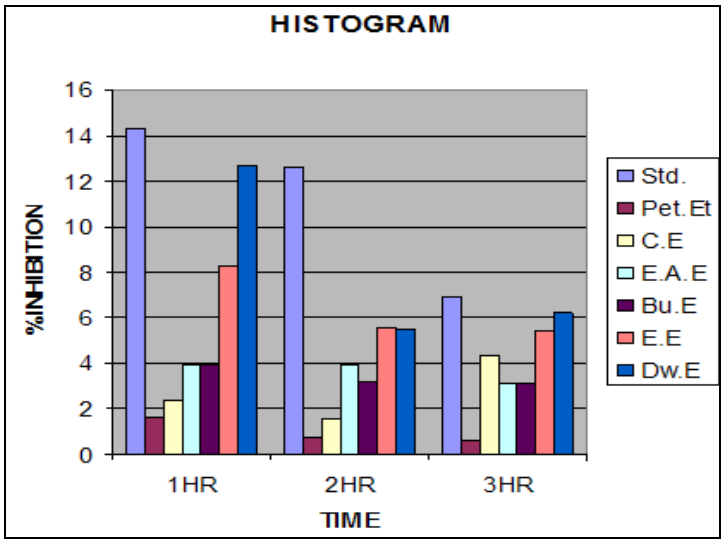

FIGURE 1: ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN INDUCED RAT PAW OEDEMA METHOD

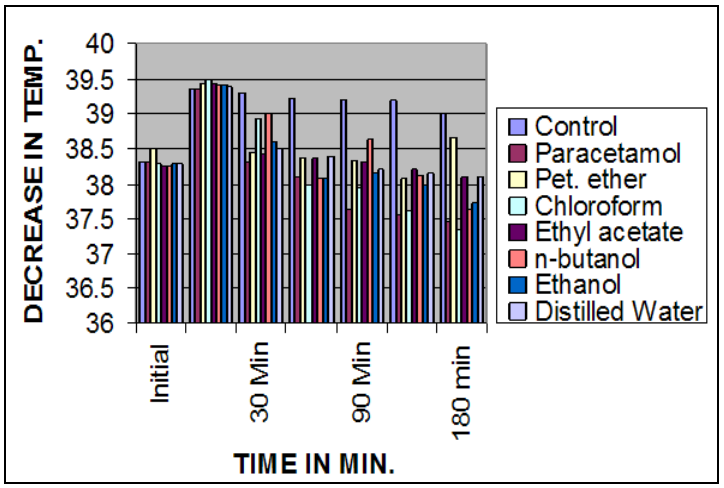

FIGURE 2: ANTIPYRETIC ACTIVITY OF VARIOUS EXTRACTS OF CALOTROPIS GIGANTEA AGAINT BREWER'S YEAST INDUCED PYREXIA IN ALBINO RATS

SUMMARY AND CONCLUSION: In the present study, leaves of *Calotropis gigantea* Linn collected and shade dried. It was reduced to required particle then subjected to the successive extraction with the Petroleum ether (60-80^{o}C), chloroform, Ethyl acetate, n-Butanol and Ethanol, distilled water in soxhlet extractor.

Some part of the total extracts was subjected to preliminary phytochemical investigation, for the identification of various Phytoconstituents and rests of extracts were utilized for pharmacological screening for assessment of Anti-inflammatory and Antipyretic activity using following method. The extracts after the preliminary phytochemical investigation have shown the presence of following active principles.

Petroleum ether extract:		Mucilage, fats
Chloroform extract	:	Glycosides, Sterols
Ethyl acetate extract	:	Sterol, Carbohydrates.
n-Butanol extract	:	Carbohydrates, Sterols
Ethanol extract	:	Sterol, Carbohydrates, Flavonoids
Distilled water extract	:	Carbohydrates, Flavonoids.

Sterols, Ethanol, chloroform and n-butanol extract were subjected to thin layer chromatography for detecting the presence of sterols and flavonoide. The results of anti-inflammatory and antipyretic activity can be summarized as under;

Carrageenan induced Rat Paw Oedema Method: The ethanol extract and distilled water extract showed good significant reduction in paw oedema as compared to control group, where as Petroleum ether (60-80^{o}C) extract, Chloroform extract, Ethyl acetate, n-Butanol has showed comparatively less significant reduction in paw oedema volume.

Yeast induced Hyperpyrexia Method: The chloroform and n-butanol extract showed good significant reduction in rectal temperature as compare to control group, where as pet. ether, ethyl acetate, ethanol and distilled water extracts showed less significant reduction in rectal temperature.

Hence, to put into the active principle of *Calotropis gigantea* linn like glycoside, sterols, carbohydrate, flavonoids, terpenoide may be responsible for anti-inflammatory and antipyretic activity.

REFERENCES:

1. Sharma PC, Yelne MB and Dennis TJ: Data base on Medicinal plant used in Ayurveda. Central Council for Research in Ayurveda and siddha, New-Delhi, Vol. I, 2002: 6-10
2. Khare CP: Medicinal plant of India An encyclopedia. Springer, New York, 2004: 119-121.
3. Akhtar H, Mishra LS, Gupta MM: Dictionary of Indian Medicinal Plant. Published by central Institute of Medical & Aromatic plant, Lucknow, 1992: 97.
4. Donna D: Nursing Herbal medicine handbook. Senior publisher, Springhouse corporation, 1990: 1-5.
5. Lombardino JG: In; Non steroidal anti-inflammatory Drugs. John wiley, and Sonc Inc. (Ltd), Newyork, 1985: 781, 116-128.
6. Jeffery AG, Charles AD: In; Alteration in body temperature. In; Harrison Principle of Internal Medicine, Mc-Grew Hill health profession division, New York, Edition 14, Vol. 1, 1988: 84-86.
7. Kokate CK: Practical Pharmacognory. Villabh Prakashan, Delhi, Edition 4, 1994: 110
8. Kokate CK and Purohit AP: Textbook of Pharmacognosy. Nirali Prakashan, Delhi, Edition 11, 1999: 138.

9. Harborne JB: In, Phytochemical method, A Guide to Modern Techniques of plant Analysis. Chapman and Hall, New York, Edition 2, 1973: 125, 196.
10. Guideline Document on Acute Oral Toxicity, Environmental Health and Safety Monograph series on Testing and assessment no. AOT 25.
11. Vogel HG: In; Drug Discovery and Evaluation, Pharmacological Assays. Springer-Verlag, Berlin Heidelberg, New York, II completely revised, Edition 2, 2002: 759.
12. Vogel HG: In, Drug Discovery and Evaluation Pharmacological Assays. Springer-Verlager Berlin Heidelberg, New York, Edition 2, 2002: 696-697.

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