A STUDY ON BIOLOGICAL ACTIVITIES AND ANTIFUNGAL ACTIVITIES OF *SARACA INDICA* L.

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Abstract

The medicinal plant *Saraca indica* L. belongs to the family Caesalpiniaceae. This plant is known as Thaw-ka in Myanmar. The specimens were collected from Dagon University, East Dagon Myothit Township, Yangon Region, during the flowering and fruiting period, January to June, 2019. In this research morphological characters, biological activities tests and antimicrobial activities tests were studied. In the morphological study, the plant was small tree, dark green leaves and grey to dark brown barks. In biological activities tests, DPPH scavenging activity, total phenolic content, total flavonoid content, ABTS radical cation decolouration assay method and total tannin content tests were carried out. In DPPH activity test, ethanol 70 % leavesextractgave the highest activity. In the ABTS test, distilled water leaves extract and ethanol leaves extract were higher than the bark extracts. Ethanol 70 % of leaves and bark extracts showed the best activity in total phenolic content. The results of total flavonoid content test, ethanol 70% of leaves extract exhibited the highest contents. The highest tannin content was found in the Ethanol 70% of bark extract. The antifungal activities were carried out by paper disc diffusion method on *Phythium graminicola.* Methanol extract showed the significant against and acetone extract showed the least activities on test microorganisms.

Keywords : Extracts, biological activities, antifungal activities

Introduction

Plants contain numerous biological active compounds, many of which have been shown antifungal properties.Plants-derived medicines have been part of traditional health-care in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases.*Saraca indica* L.belongs to the family Caesalpiniaceae. *Saraca indica* L. is used in many pharmacological activities like anti-menorrhagic, anti-cancer, anti-oxytoxic, anti-inflammatory, anti-ulcer,antimicromicrobial activity. Mainly contains glycoside, flavonoids, tannins, saponins, esters and primary alcohols.The plant has been greatly used as traditional medicine for women related problems such as menorrhagia, leucorrhoea, bleeding hemorrhoids, dysfunctional uterine bleeding etc (Mohammad *et al*., 2017). Leaves are useful in stomach pain, help to remove worms from the stomach and thus provide relief from pain and swelling.

Parts of the tree used in traditional Ayruvedic medicine and homeopathic therapies. Juice obtained from boiling of bark said to be effective against female medicinal disorders like menstrual irregularities.Flowers are eaten against dysentery.Bark is used to prepare cosmetics that help to improve skin complexion. The bark of the tree also has anti-fungal, anti-bacterial and pain relieving properties.The bark decoction helps to treat internal piles. Flowers controls blood loss in stools. Seeds powder helps to control kidney stones. Fruits are used as a masticating kidney stones and a replacement for betel nuts.The wood is used in the buildings, the fruit as fodder and from the flowers get a dye. The aim and objectives

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of this research are to verify the morphological characters of this plant, to detect the biological activities of plant and to examine the antimicrobial activities of plant.

Materials and Methods

Collection of plant samples

The fresh leaves and barks were collected from Dagon University, East Dagon Township, Yangon Region.They were collected especially during the flowering and fruiting period from January to June in 2019. The collected fresh specimens of both vegetative and reproductive parts of the plants were identified by using literatures of Lawrences, 1964; Backer, 1965; Hundley and Chit Ko Ko, 1987; Dassanayake, 2000 and Kress *et al.*, 2003.

Preparation of powder samples

The collected samples of leaves and barks were washed with water and cut slices by knife. These slices were initially allowed to air-dry in the lab for two weeks and then finally ground into a fine powder using a motor and pestle.

Extraction of samples

The leaves and barks powder were soaked in different composition of various solvents such as ethanol, methanol, acetone and distilled water for about three days and thoroughly shake. Then, the mixtures were filtered using Whatman No.1 paper and stored at refrigerator.

Biological activities tests

(1) DPPHscavenging activity

The electron donating ability (EDA) of the extract was measured for reducing power of each extract as an electron donating effect according to the method of Blois (1958). In other words, 0.9 mL of 0.1 mM DPPH (1-1-diphenyl-2-picrylhydrazyl) and 80 μL of extraction solvent were added to 20 uL of extract to make the total volume 1 mL. The reaction solution is mixed for about 10 seconds and left at room temperature for 10 minutes. The reaction solution was measured for absorbance at 516 nm (Spectramax M2, Molecular Devices, Sunnyvale, Calif., USA). Electron donating ability is expressed as a percentage through the absorbance of the addition and non-addition of the extract.

$$EDA (\%) = (1 - A / B) \times 100$$

A: absorbance of extract addition

B: absorbance of extract-free

(2) ABTSradical cation decolouration assay

Antioxidant activity using ABTS radical was measured by ABTS radical cation de colouration assay method. 7.4mM 2,2'-Azino-bis-3

ethylbenzobenzothiazoline-6-sulfonic acid (ABTS) and 2.45 mM potassium persulfate were mixed at the final concentration for 24 hours in the dark at room temperature to form ABT cation, followed by 700 nm Dilute with distilled water so that the absorbance value at 1.4 ~ 5. 100 mL of methanol extract was added to 2 mL of diluted ABTS solution, and then reacted with an absorbance of 700 nm exactly 5 minutes. The ABTSradical scavenging activity was calculated by the following equation.

ABTS radical scavenging activity (%) = [1− (sampleabsorbance / controlabsorbance)] × 100

(3) Total polyphenol content

The total polyphenol content was analyzed based on the principle that the Folin-Ciocalteu reagent was colored by molybdenum blue as a result of the reduction of the Folin-Ciocalteu reagent by the polyphenolic compound of 80% methanol extract according to the method of Dewanto *et al*. (2002). That is, 2 mL of 2% Na_2CO_3 solution was added to 100 μL of each extract, and the mixture was left to stand for 3 minutes, and 100 μL of 50% Folin-Ciocalteu reagent was added. After 30 minutes, the absorbance of the reaction solution was measured with a spectrophotometer at 700 nm. Using garlic acid (SigmaChemical Co.) as a standard, the total polyphenol content was expressed as mg gallic acid equivalents in g of the sample after the correction line (y = 0.0018x-0.0327; R2 = 0.9991) was prepared.

(4) Total flavonoid content

The total flavonoid content was determined by Choi *et al*. (2010) and 1 mL of distilled water and 5% $NaNO_2$ were added to 75μL of methanol extract, followed by 10% $AlCl_3$, $6H_2O$ 150 uL for 5 minutes. It was left to stand and 500NuL of 1N NaOH was added. After 11 minutes, the absorbance value of the reaction solution was measured with a spectro-photometer at 500 nm. Using the (+)-catechin (Sigma Chemical Co.) as a standard, the total flavonoid content was corrected (y = 0.004x−0.0912; R_2 = 0.9993) and expressed as mg catechin equivalents in g of the sample.

(5) Total tanin content

Total tannins were determined based on the method described by Makkar *et al*. (2007) which compares the TPC extracts treated with polyvinylpyrrolidone (PVPP) with those untreated. Tannins, being protein-binding phenolic compounds, would bind to the PVPP.

The TPC was determined via the Folin-Ciocalteau method as detailed previously. For PVPP treatment, 1 mL of distilled water was added to 100 mg PVPP before adding 1 mL of extract. The mixture was vortexed, incubated at 4°C for 15 min, vortexed again and centrifuged at 3000 g for 10 min. The supernatant (consisting of simple phenolics other than tannins) was collected and the phenolic content was determined using the TPC assay. Results were expressed as mg equivalent per 100 g sample (mg TAE/ 100 g).

Antifungal activities test

For antifungal activities were tested by 70 % of ethanol, methanol, acetone, watery extract and distilled water of leaves extracts on the test organism by paper disc diffusion methods according to Davis *et al*., (1971). The fungus *Pythium graminicola* KACC 40155 was obtained from Department of Agriculture Biology, Jeonbuk National University (JBNU).

Results

Figure (1) Morphological Characters of *Saraca indica* L.

Table (1) Extraction of *Saraca indica* L. Barks.

No.	Solvents	%	Bark (20 g/ 100 mL)		Leaves (50 g/ 50 mL)	
			Weight (g)	**Extract (mL)**	**Weight**	**Extract(mL)**
1	Ethanol	50	17.11	52.50		
		70	17.33	66.00	8.87	65.00
		90	17.44	50.00		
2	Methanol	50	17.37	42.50		
		70	17.07	42.50	8.72	42.50
		90	17.44	36.00		
3	Acetone	50	19.97	37.50		
		70	19.07	27.70	8.25	25.00
		90	17.46	27.45		
4	N-Butanol	50	19.04	57.00		
		70	19.03	61.50	9.66	60.00
		90	19.11	77.50		
5	Dimethyl sulfoxide	50	32.65	77.50		
		70	35.31	80.00	22.90	77.50
		90	38.28	80.00		

Table (2) Extraction of *Saraca indica* L. Leaves

Solvents	%	Powder weight	Dry weight	Loss weight	Extract(ml)		mg/ml	μ l
EtOH	70	10	8.3	1.7	65.0	0.026153	26.153	38.2365
MeOH	70	10	7.9	2.1	42.5	0.049411	49.411	20.2384
Ace	70	10	7.8	2.2	25.0	0.088	88.0	11.3636
N-BtOH	70	10	9.0	1.0	60.0	0.016667	16.667	59.9988
DMSO	70	10	6.9	3.1	77.5	0.04	40.0	25.0
Water		50	41.20	8.8	137.5	0.064	64.0	15.625

Table (3) Extraction by hot water

Solution	Bark (100 g/ 500 mL)		Leaves (50 g/ 50 mL)	
Hot Water	Weight (g)	Extract (mL)	Weight(g)	Extract(mL)
	88.81	194.5	43.32	137.5

Biological activities of *Saraca indica* L.

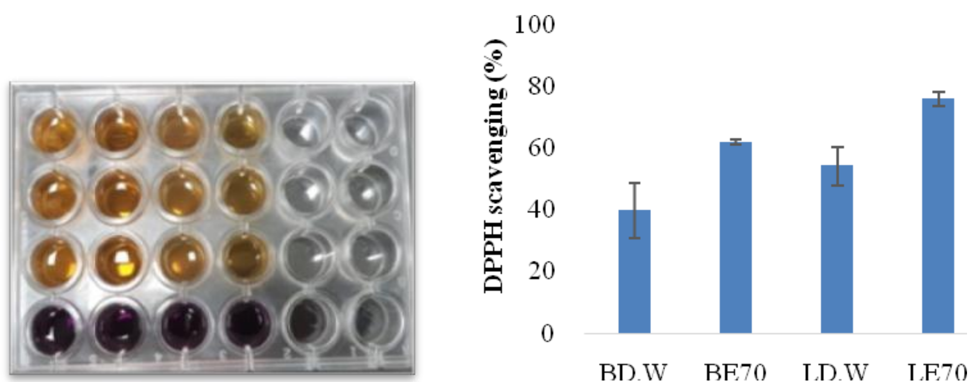


Figure (2) DPPH Scavenging Activity of *Saraca indica* L. Barks and Leaves

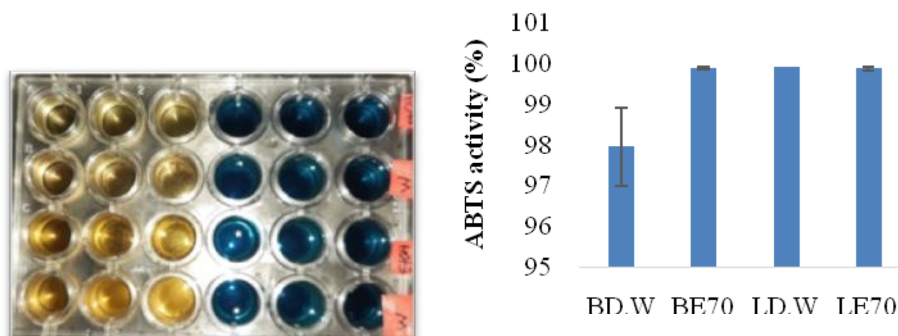


Figure (3) ABTS Activity of *Saraca indica* L. Barks and Leaves

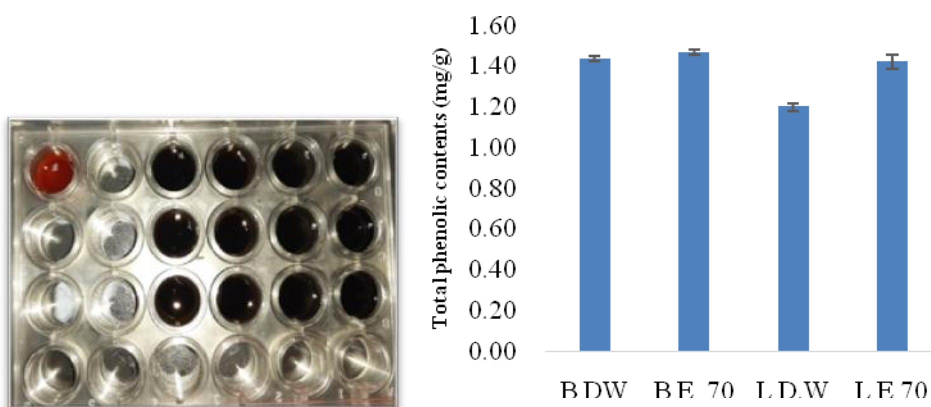


Figure (4) Total Phenolic Content of *Saraca indica* L. Leaves

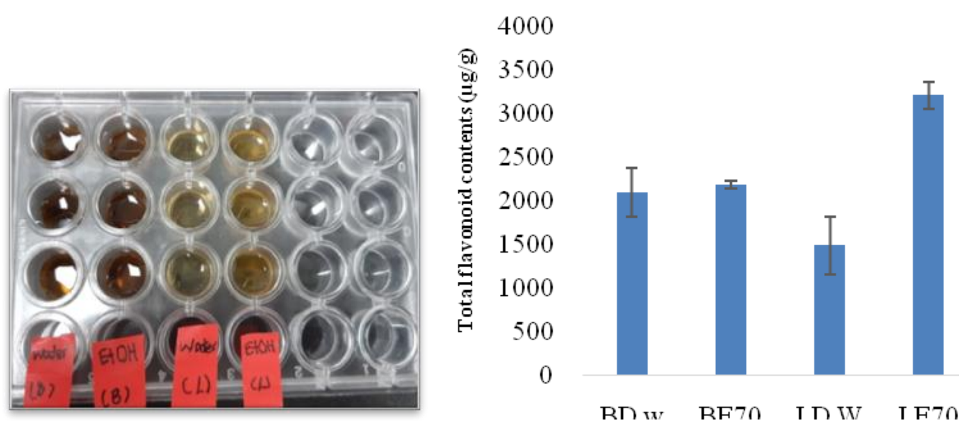


Figure (5) Determination of Total flavonoid content of *Saraca indica* L. Leaves

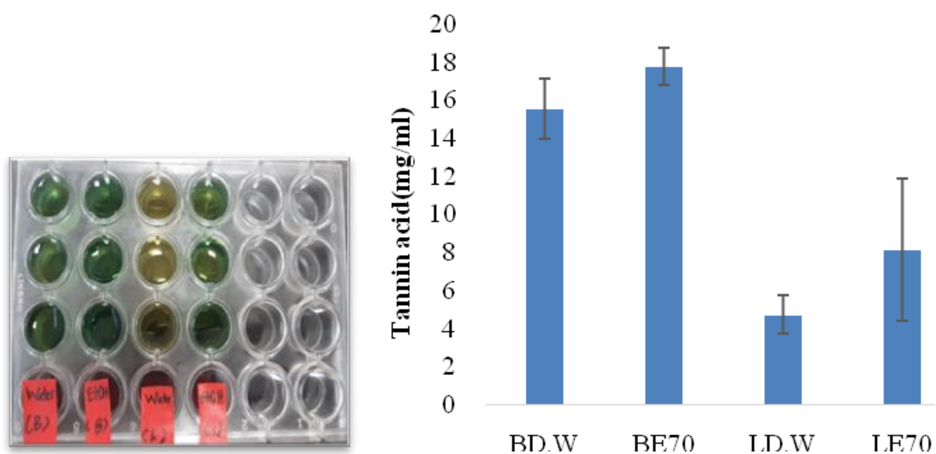
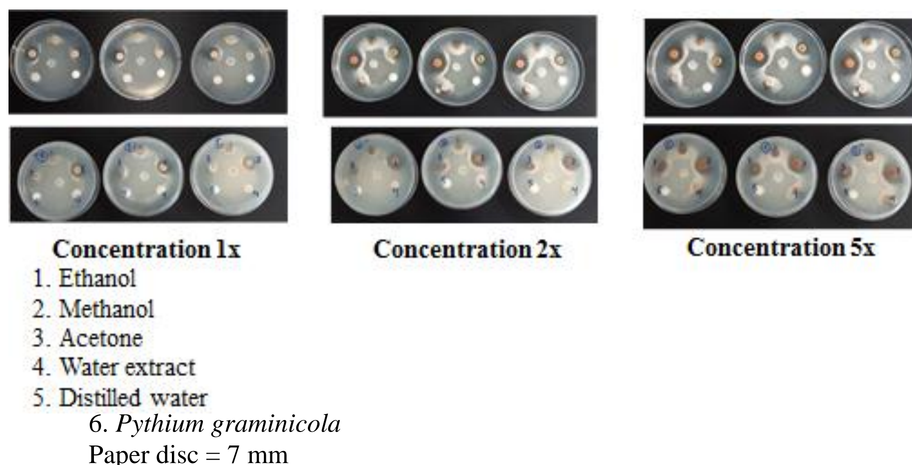


Figure (6) Determination of Standard Material Tannin acid of *Saraca indica* L.

Antifungal Activities of Leaves

In the present study, methanol leaves extract showed the significant against on test organism and acetone leaves extract showed the least activity on test organism, fungus *Pythium graminicola* (Table 4 and Figure 7).



6. *Pythium graminicola*
Paper disc = 7 mm

Figure (7) Antifungal Activities of *Saraca indica* L. Leaves

Table(4)Inhibition Zones Exhibited by Different Solvents

Extracts / Conc:	EtOH	MeOH	Ace	Watery Extract	Pure DW
1x	_	18mm	12mm	_	_
2x	_	18mm	12mm	_	_
5x	_	25mm	15mm	_	_

Discussion and Conclusion

The plant *Saraca indica* L. belongs to the family Caesalpiniaceae and small trees. The leaves are alternate, paripinnately compound, petiolate and stipulate. Leaflets are elliptic-lanceolate, rounded at the base, margin entire, acuminate apex, both surfaces shinning, dark green. The stipules are lanceolate, caducous. The inflorescences are axillary, corymb racemose, peduncles cylindrical, reddish green and glabrous. The flowers are yellow to red, bracteate, bracteolate, pedicellate, complete, bisexual, regular, actinomorphic, tetramerous and hypogynous. The sepals are yellowish to red. The petals are lacking. The stamens are fused. The filaments are filiform and distinct. The anthers are dithecous. The ovary is superior and oblong. This data was agreed with Dassanayake, 1991.

The primary benefit of *Saraca indica* L. is a brain tonic and improves memory and intellect. It can be used to treat epilepsy and headache. It also controls vomiting and helps to cure diabetes. The herb of this tree can act on uterine muscles and endometrium and thus provides relief from abdominal pain and other spasms. It also helps to treat irregular menstrual cycles, amenorrhea, leucorrhea, fibroids, cysts and other related disorders. This tree is widely used to treat gynecological and menstrual

problems in women and it helps to remove toxins fromour blood and therefore provides excellent benefits for our skin. The flowers were eaten by cooked, aromatic, with a somewhat sour flavour. Eaten as potherb. Fruits are used as masticatons as a replaced for betel nuts. Although the health benefits of this tree are numerous, pregnant women should abstain from consuming products from this tree as it might lead to complications.

According to present research, in DPPH scavenging activity test, the ethanol extracts of leaves and barks were higher than the watery extracts of leaves and barks. In ABTS test, watery extract of leaves, ethanol extracts of leaves and barks were higher than watery extract of barks. In total phenolic content, watery extract of barks, ethanol extracts of leaves and barks were higher than watery extract of leaves. In total flavonoid content, ethanol extract of leaves higher than watery extract of barks, the ethanol extract of barks and leaves. In total tannin content, ethanol extract of barks showed the highest and watery extract of leaves showed the lowest content. In the antifungal activities tests, methanol extract showed significant against on fungus *Pythiumgraminicola*.Theseresults were accordance with the Mohammad (2017).The current investigation proved the antifungal efficacy of the plant. The traditional use of the plant can cure against different diseases.Further principles present in the extracts which could possibly be exploited for pharmaceutical use.

Acknowledgements

I would like to express my sincere gratitude to Professor Dr. Bang Keuk-Soo, Project Manager, Jeonbuk National University, and professor Ju, Ho-Jong, Department of Agriculture Biology for support ,expert guidance, immense knowledge throughout my study and research. I would like to express my sincere gratitude to Professor Dr. Myat Myat Moe, Head of the Botany Department,Dagon University, for allowing me to conduct research in JBNU in Korea. I am also grateful to my colleague Mr. Hyun-Gi and Mr. Thang Tung Lian, his teaching and guiding for experiments.Finally, I want to express my special thanks to members of the South Korea JBNU –Project.

References

Aditya Mishra, Anil Kumar, Neelam Rajbhar and Ashok Kumar (2013) ***International Journal of Pharmaceutical and Chemical Sciences Vol.2***

Antonio Cano and Marino B. Arnao (2018). **Measurement of Antioxidant Activity & Capacity** Determent of Plant Biology, Faculty of Biology, University of Murcia, Murcia, Spain**.**

Atlas (1993), ***Handbook of Microbiology Media***, CRC Press, London

Backer, C.A. 1968, ***Flora of Java,*** Vol. Ill, Wolters - NoordholT N.V. - Groningen. The Nether Lands.

Blois MS. Antioxidant determinations by the (1958). Use of a stable free radical. Natuure. 181:1199-1200. doi: 10.1038/1811199aO.

British Pharmacopoeia, 1968, The Pharmaceutical Press, London and Bradfod.

Central Council for Research in Unani Medicine, 1987, Physicochemical Standard of Unani Formation, India, New Delhi, Ministry of Health and family Walfare.

Choi HK, Yoon JH, Kim YS and Kwon. DY. (2010). **Japan Society for Bioscience, Biotechnology and Agrochemistry,** Volume 74, Issues 9-10, The Ohio State University.

Cruikshank, R.T.P., 1975, ***Medicinal Microbiology,*** 11th ed., H and S Living Stone Ltd. Ediburge and London.

Dassanayake, M.D. and W.D. Clayton, 2000, **Flora of Ceylon,** Vol-XIV, A.A. Balkema/ Rotterdom/ Bookfield.

Davis, W. W. T. R. Stout, (1971). **Disc Plate Method of Microbiological Antibiotic Assay.** Applied Microbiology Vol 22, No.4

Dewanto, V., Wu, X., Adom, K. K. Liu, R. H. (2002).**Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity.** *Journal of Agricultural and Food Chemistry*, **50**, 3010-3014

Dubey, R. C. and D. K. Mabeshwari, D. K., 2002. **Practical Microbiology. S.** Chand and Company Ltd. Ram Nagar, New Delhi, 110055

Hundley H.G. and Chit Ko Ko, **1987,** ***List of Trees, Shrubs, Herbs and PrincipalClimbers*, etc,** Government Printing Press, Yangon.

Lawrence, G.H.M., 1964, ***Taxonomy of Vascular Plants,***9th Ed., The Macmillan Company, New York, London.

Makkar, H.P.S.; Siddhuraju, P.; Becker, K. **Plant Secondary Metabolites**; Humana Press: Totowa, NJ, USA, 2007; pp.67-82

Marini Bettolo, G. B; Nicolettic, M., and Patamia, M., 1981, ***Plant Screening by Chemical Chromatographic Procedure Under Field Conditions Journal of Chromatography.***

Mohammad Abu Bin Nyeem, 2017. ***National Journal of Advanced Research*,** Vol.3; Issue 2; May 2017, pg. 03-07

Robinson, T., 1983, ***The Organic Constituents of Higher Plants*,** Department of Biochemistry, University of Massachusetts.

Tin Myint and Myo Khin (1996), ***Floristic Survey of Yangon University Estate*** (Diamond Jubilee)

Trease and Evans, 2002, ***Pharmacognosy,***15th ed. W.B. Saunders, Edinburgh London New York Philadelphia St Louis Sydney Toronto.

Vogel, A.I., 1956, ***A Text book of Practical Organic Chemistry*,** Longmans Green & Co., Ltd. London.