World Journal of Clinical Pharmacology, Microbiology and Toxicology
World J. Clin. Pharmacol. Micrbiol. Toxicol Vol 1 [4] November 2015: 22-29
Online ISSN 2454-1729
Journal's URL: http://wjcpmt.com
Impact factor [JIF] : 1.05
Global Impact Factor: 0.384

ORIGINAL ARTICLE

Pharmacognostical and Phytochemical Studies on Barks of Moraceae Trees

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ABSTRACT

Bark is the outermost layers of stems and roots of woody plants. Plants with bark include trees, woody vines, and shrubs. Bark refers to all the tissues outside of the vascular cambium and is a nontechnical term. Products used by people that are derived from bark include: bark shingle siding and wall coverings, spices and other flavorings, tanbark for tannin, resin, latex, medicines, poisons, various hallucinogenic chemicals and cork. Bark has been used to make cloth, canoes, and ropes and used as a surface for paintings and map making. A number of plants are also grown for their attractive or interesting bark colorations and surface textures or their bark is used as landscape mulch. It serves as protection against damage from parasites, herbivorous animals and diseases, as well as dehydration and fire. Cork can containantiseptics like tannins, that protect against fungal and bacterial attacks that would cause decay. So we were very much interested to study the barks of moraceae family, they were Artocarpus heterophyllus Lam., Ficus bengalensis L., Ficus racemosa L., Ficus religiosa L. and Morus nigra L., The bark containing phytochemical compounds, pharmacognostical behavior and mycoflora were screened in this study.

Keywords: Ficus Sp., pharmacognostical behavior

CITATION OF THIS ARTICLE
Venkatraman, A., Krishnaveni, M. and Suresh Kumar, J.Pharmacognostical and Phytochemical Studies on Barks of Moraceae Trees. *World J. Clin. Pharmacol. Microbiol.Toxicol. Vol 1 [4] November 2015: 22-29*

INTRODUCTION

Medicinal plants have a long-standing history in many indigenous communities and continue to provide useful tools for treating various diseases. Medicinal plants are moving from fringe to main stream use with a greater number of people seeking remedies and health approach (Saha *et al.*, 2010). Recent work revealed the potential of several herbs as sources of drugs (Iwu, 2002). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

Endophytes are microbes that colonize the living internal tissues of plants without causing any immediate overt negative effects(Bacon and White, 2000). They are a largely unexplored component of biodiversity, especially in the tropics. Endophytic fungi have been isolated from leaves, stems and roots of woody plants in the temperate regions and the tropics(Rodrigues, 1994: Wilson and Carroll,1994: Frohlich and Hyde, 1999). They have a protective role against insect herbivory and many are potential producers of novel antimicrobial secondary metabolites (Arnold,*et al.*, 2001). Endophytes are constantly exposed to intergeneric genetic exchange with the host plant. Isolation of a potent anticancer agent, taxol from *Pestalotiopsis microspora*, an endophyte of the yew tree and the phytohormone-producing fungus from rice plant, *Gibberella fujikuroi* suggests the potential of endophytes as a source of useful metabolites (Strobel *et al.*, 1998: Stierle *et al.*, 1993).

The process of standardization can be achieved by stepwise pharmacognostic and phytochemical studies. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001). The need of the hour is to screen a number of medicinal plants for promising biological activity. These are chemically diverse compounds as secondary metabolites (Kordono *et al.*, 1990) and which include such well known substances such as alkaloids, glycosides, terpenes, sterols, tannis, flavanoids, phenols and resins etc. Many plants of this genus are used in medicine for the treatment of skin diseases, enlargement of liver

and spleen, dysentery, diarrhoea, diabetes, leprosy, lung complaints, leucorrhoea, heart diseases, cough, asthama, piles, ulcers, gonorrhea and rheumatism (Anonymous, 2012).

There is no scientific data available for pharmacognostical and bark moraceaes trees.The selected medicinal plants have strong background on medicinal properties. So we are interested to study the efficiency of plant of these selected plants. The main objectives of our studies were, Pharmacognostical behaviour, qualitative phytochemical screening and mycofloraof five selected barks.

MATERIALS AND METHODS

Collection of plants

The plants *Artocarpus heterophyllus, Ficus bengalensis, Ficus racemosa, Ficusreligiosa, Morusnigra* were collected from Aranji village of Tiruvannamalai District, Tamilnadu, India and the collected plants were carefully examined and identified with the help of Regional Floras (Gamble,1975; Henry *et al.,* 1983; Mathew, 1983).

Extraction of plant material

Various extract of the plants were prepared according to the methodology of Indian Pharmacopeia (Anonymous, 1966). From these extracts qualitative phytochemical analyses were done by using the procedures of (Kokate, 1995). Alkaloids, carbohydrates, tannins and phenols, flavonoids, gums and mucilage's, phytosterol, proteins, fixed oils and fat, volatile oil and saponins were qualitatively analyzed and also the Pharmacognostical studies such as total ash, water soluble ash, acid soluble ash.

Any scientific evaluation procedure/component is subjected to clear authentic fication and identification. In this research, once again, scientific authentic fication was carried out based on macroscopical as well as microscopical character observation through pharmacognostical studies, Determination of alcohol Soluble extractive, determination of water soluble extractive, determination of crude fiber content by dutch processand microbial analysis. Microbial analysis were done by standard plate count method.

The total numbers of population are enumerated individually for fungi and bacteria by taking the average of the two dilutions employed and expressed 1gm of plant material.

Table 1: Pharmacognosticalbehaviour of selected barks

Treatment	AH	FB	FRA	FRE	MN
Foreign organic matter of powdered bark	0.7	1.4	2.26	1.28	0.9
Water soluble extractive value (%)	12.67	17.27	11.27	16.38	13.09
Alcohol soluble extractive value (%)	10.24	16.28	8.48	13.88	11.20
Water soluble Ash (%)	26.42	3.6	23.54	1.25	2.30
Acid insoluble ash (%)	31.63	1.3	35	0.92	20.13
Total Ash (%)	14.68	12.5	16.3	10.23	18.09

AH - *Artocarpus heterophyllus*
FB - *Ficus bengalensis*
FRA - *Ficus racemosa*
FRE - *Ficus religiosa*
MN - *Morus nigra*

Table 2: Powder behaviour with different chemical reagents in normal visible light.

Treatment	AH	FB	FR	FR	MN
Drug powder	Dark brown	Dark brown	Light brown	Brown	Light yellow
Conc.Sulphuricacid	Black	Brown	Brown	Black	Brown
Con. H_2SO_4+water	Black	Green	Green	Dark green	Light brown
Con. HCl	Green	Black	Black	Green	Light yellow
Con. HCl +H_2O	Light green	Green	Brown	Brown	Brown
Con. Nitric acid	Black	Orange	Black	Yellow	Brown
Methanol	Light brown	Dark brown	Light brown	Dark brown	Dark yellow
Chloroform	Green	Green	Green	Green	Light green
Petroleum ether	Green	Dark brown	Green	Green	Brown
Dist. Water	Brown	Dark brown	Brown	Dark brown	Dark yellow
10% NaOH	Light yellow	Dark brown	Brown	Dark brown	Dark yellow

5% Iodine	Brown	Dark brown	Light brown	Brown	Light yellow
Picric acid	Black	Black	Black	Black	Dark brown
$FeCl_3$ solution	Red	Green	Yellow	Brick	Green
Ammonia solution	Light brown	Dark brown	Light brown	Light brown	Yellow

AH - *Artocarpus heterophyllus*
FB - *Ficus bengalensis*
FRA - *Ficus racemosa*
FRE - *Ficus religiosa*
MN - *Morus nigra*

Table 3: Preliminary phytochemical screening test of *Artocarpus heterophyllus* bark.

Test	EXTRACTS				
	Petroleum Ether Extract	**Benzene Extract**	**Chloroform Extract**	**Ethyl acetate extract**	**Methanol Extract**
1)Alkaloids					
Hager's reagent	+	+	+	-	+
Wagner's reagent	+	+	-	-	+
Dragendorff's reagent	+	-	-	-	+
2) Phenolic compounds and Tannins					
Ferric Chloride solution	+	+	+	+	+
Lead acetate test	-	+	-	+	-
Gelatin Solution	+	-	-	-	-
3) Flavonoids					
Lead acetate test	+	-	+	-	+
Sodium hydroxide test	+	+	+	+	+
Magnesium ribbon test	-	-	-	-	-
Ammonia test	+	+	+	+	+
4) Glycosides					
Legal test	+	+	+	-	+
Borntrager'stest	+	+	-	-	+
5) Carbohydrate					
Molischs'reagent	+	-	+	-	+
6)Portions and Amino acids					
Ninhydrin reagent	-	-	+	-	+
Biuret reagent	-	+	-	+	-
Millon's reagent	-	+	-	-	-

Table 4: Preliminary phytochemical screening Tests of *Ficus bengalensis* bark.

Test	EXTRACTS				
	Petroleum Ether Extract	Benzene extract	Chloroform Extract	Ethyl acetate extract	Methanol Extract
1)Alkaloids					
Hager's reagent	+	+	+	+	+
Wagner's reagent	+	-	+	+	-
Dragendorff's reagent	-	-	-	-	-
2) Phenolic compounds and Tannins					
Ferric Chloride solution	+	-	-	+	+
Lead acetate test	+	+	-	-	+
Gelatin Solution	-	-	-	+	-
3) Flavonoids					
Lead acetate test	-	+	+	+	-
Sodium hydroxide test	+	-	-	+	+
Magnesium ribbon test	-	-	-	-	-
Ammonia test	+	+	+	-	-
4) Glycosides					
Legal test	-	-	+	-	-
Borntrager'stest	+	-	+	-	+

5) Carbohydrate					
Molischs'reagent	+	-	+	+	-
6)Portions and Amino acids					
Ninhydrin reagent	+	+	-	+	-
Biuret reagent	+	-	-	-	-
Millon's reagent	-	-	-	-	-

Table 5: Preliminary phytochemical screening tests of *Ficus racemosa* bark.

Test	EXTRACTS				
	Petroleum Ether Extract	Benzene Extract	Chloroform Extract	Ethyl acetate extract	Methanol Extract
1)Alkaloids					
Hager's reagent	-	-	-	-	-
Wagner's reagent	+	-	-	-	+
Dragendorff's reagent	-	-	-	-	+
2) Phenolic compounds and Tannins					
Ferric Chloride solution					
Lead acetate test	-	-	+	-	+
Gelatin Solution	+	-	+	-	-
	-	-	-	-	+
3) Flavonoids					
Lead acetate test	-	+	-	+	+
Sodium hydroxide test	-	-	+	-	-
Magnesium ribbon test	-	+	-	+	+
Ammonia test	-	+	+	+	-
4) Glycosides					
Legal test	+	-	+	+	+
Borntrager'stest	-	-	-	-	-
5) Carbohydrate					
Molischs'reagent	-	+	-	-	+
6)Portions and Amino acids					
Ninhydrin reagent	-	-	+	-	+
Biuret reagent	-	-	+	-	-
Millon's reagent	+	+	-	-	+

Table 6: Preliminary phytochemical screening tests of *Ficus religiosa* bark.

Test	EXTRACTS				
	Petroleum Ether Extract	Benzene extract	Chloroform Extract	Ethyl acetate extract	Methanol Extract
1)Alkaloids					
Hager's reagent	+	+	+	+	-
Wagner's reagent	+	-	-	-	+
Dragendorff's reagent	+	+	+	-	+
2) Phenolic compounds and Tannins					
Ferric Chloride solution					
Lead acetate test	+	-	+	+	-
Gelatin Solution	+	+	+	+	+
	-	-	-	-	-
3) Flavonoids					
Lead acetate test	+	+	+	-	+
Sodium hydroxide test	+	-	+	-	+
Magnesium ribbon test	+	-	+	-	+
Ammonia test	+	-	+	-	+
4) Glycosides					
Legal test	+	-	-	-	+
Borntrager'stest	-	-	-	-	-
5) Carbohydrate					

Molischs'reagent	+	-	+	+	-
6)Portions and Amino acids					
Ninhydrin reagent	-	-	+	-	+
Biuret reagent:	-	+	+	+	+
Millon's reagent	+	+	+	+	+

Table 7: Preliminary phytochemical screening tests of *Morus nigra* bark.

Test	EXTRACTS				
	Petroleum Ether Extract	Benzene extract	Chloroform Extract	Ethyl acetate extract	Methanol Extract
1)Alkaloids					
Hager's reagent	-	+	-	+	-
Wagner's reagent	-	+	+	+	-
Dragendorff's reagent	-	-	-	+	+
2) Phenolic compounds and Tannins					
Ferric Chloride solution	+	-	-	+	-
Lead acetate test	-	-	-	+	-
Gelatin Solution	+	-	-	-	+
3) Flavonoids					
Lead acetate test	-	-	+	-	+
Sodium hydroxide test	+	-	+	-	-
Magnesium ribbon test	-	+	-	-	+
Ammonia test	+	-	+	+	-
4) Glycosides					
Legal test	-	+	-	-	-
Borntrager'stest	+	-	+	-	+
5) Carbohydrate					
Molischs'reagent	+	+	+	+	+
6)Portions and Amino acids					
Ninhydrin reagent	+	-	-	-	+
Biuret reagent	+	+	-	+	-
Millon's reagent	+	-	-	-	-

Table 8: Mycofloraaverage on selected barks.

S.No	Name of The bark Plant	Dilution factor				Average
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	
1	*Artocarpus heterophyllus*	198	208	212	68	171.5
2	*Ficus bengalensis*	211	229	242	92	193.5
3	*Ficus racemosa*	138	156	198	56	137
4	*Ficus religiosa*	192	204	211	88	173.75
5	*Morus nigra*	116	122	163	59	115

Table 9: Mycoflora of selected barks.

S.No	Name of the Fungal strains	**AH**	**FB**	**FR**	**FR**	**MN**
1	*Aspergillus flavus*	+	+	+	-	+
2	*Aspergillus fumigates*	+	+	+	+	-
3	*Aspergillus niger*	+	+	+	+	+
4	*Fusarium (sps)*	-	-	+	+	-
5	*Penicillium (sps)*	+	+	+	+	+
6	*Alternaria alternate*	+	+	-	+	-
7	*Trichoderma harianum*	-	+	+	+	+
8	*Chaetomium globosum*	+	+	+	+	-
9	*Curvularia lunata*	+	+	+	+	+
10	*Dactylosporium macropus*	+	+	-	+	-
11	*Humicola fuscoatra*	-	+	+	+	+
12	*Mucor mucedo*	+	+	+	+	-
13	*Torula herbarum*	+	+	+	+	+
14	*Trichoderma viride*	+	+	-	+	-

AH - *Artocarpus heterophyllus*
FB - *Ficus bengalensis*
FR - *Ficus racemosa*
FR - *Ficus religiosa*
MN - *Morus nigra*

RESULTS AND DISCUSSION

The family *Moraceae* was named after the mulberry. The genus *Artocarpus* includes the breadfruit and jackfruit. Species of the diverse fig genus *Ficus,* take an assortment of forms. Some of the more unusual species include the strangler figs such as the banyan tree (*Ficus benghalensis*), which begins life as an epiphyte and sends down rope-like roots that eventually encircle and kill the host tree. The banyan tree is native to India, where it is considered sacred by Hindus. The Bo Tree, (*Ficus religiosa*), of India is believed to bring wealth and happiness to its owners. (Duncan, 1988; Everett, 1968; Godfrey, 1988).

Hence, it is very difficult to identify the original from the adulterants/substitutes while procuring crude drug from the market.

The plant containing phytochemical compounds, pharmacognostical behaviors and mycoflora were screened from the bark of *Artocarpus heterophyllus* Lam., *Ficusbengalensis* L., *Ficusracemosa* L., *Ficusreligiosa* L. and *Morusnigra* L., Mycoflora of our five bark were isolated and identified and after they were tabulated. Pharmacognosticalbehaviour of powder with different chemical reagents were screened on normal visible light and UV light. The solubility percentages of bark powder with different organic solvents were recorded.

Tree bark is very complex in structure and has the potential of containing many primary and secondary metabolites. Products stored in the bark are useful for preparation of many drugs. The complex structure of the bark can be utilized for botanical identification to maintain the quality and purity of the drug (Brinda *et al*., 2000). *Nalpamaram* is an important group of Ayurvedic formulation that constitutes the barks of *Ksirivrksas* (4 laticiferous tree species), namely *Ficus racemosa*, *Ficus virens*, *Ficus religiosa* and *Ficus benghalensis*, widely used in the treatment of skin diseases with *pitta* and *rakta* predominance and also used in various ailments (Sivarajan and Balachandran, 1994; Joy *et al.,* 2001).

Barks of some of these 4 species, such as *Ficus virens*, are also equated with many other species like *Ficus microcarpa* L. f. *Ficus infectoria* Roxb., *Ficus arnottiana* Miq, *Ficus lacor* Buch-Ham and *Ficus talboti* King (Nadkarni, 1954; Singh & Chunekar, 1972; Kapoor & Mitra, 1979; Sharma, 1983). Hence, it is very difficult to identify the original from the adulterants/substitutes while procuring crude drug from the market. The barks of 4 *Ficus* species contains tannin, wax, saponin gluanol acetate, *β*-sitosterol, leucocyanidin- 3-O-*β*-D - glucopyrancoside, leucopelargonidin-3-O-*β* -D - glucopyranoside, leucopelargonidin-3-O-α-L-rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, α-amyrin acetate, leucoanthocyanidin, and leucoanthocyanin (Husain *et al*., 1992).

The pharmacognostical behaviour, foreign organic matter was high in *Ficus racemosa* (2.26%) and lower 0.7% in *Artocarpus heterophyllus*. The solubility extractive value percentage was maximum 17.27% in *Ficus bengalensis* and minimum 12.67% in *Artocarpus heterophyllus*. Alcohol soluble extractive range between 8.48 and 16.28%. The water soluble ash percentage was in between 1.25- 35%. Acid insoluble ash percentage lie between 1.3-31, 63%. Total ash content was elevated stage in *Morus nigra* (Table - 1).Powder behaviour with different chemical reagent were mixed with our selected bark powder and after they emitted different colours were recorded in normal and UV light (Table-2). *Artocarpus heterophyllus* bark powder contains phytochemicals were screened through such tests, they gave maximum positive results in alkaloid and flavonoids but minimum in proteins and amino acids (Table - 3).*Ficus bengalensis* bark powder contains phytochemicals were screened through such tests,they gave maximum positive results in alkaloid and flavonoids but minimum in proteins and amino acids (Table - 4). *Ficus racemosa* bark powder contains phytochemicals were screened through such tests, they gave maximum negative results in alkaloid but minimum in flavonoids, proteins, amino acids glycosides (Table - 5). *Ficus religiosa* bark powder contains phytochemicals were screened through such tests, they gave maximum positive results in alkaloid and flavonoids but minimum in proteins and amino acids (Table - 6). *Morus nigra* bark powder contains phytochemicals were screened through such tests, they gave maximum negative results in alkaloid and flavonoids but minimum in proteins and amino acids (Table - 7).The bark containing mycoflora were screened they serial dilution factor such an 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}. Among the all bark materials *Ficus bengalensis* bark containing mycoflora average was 193.5 and minimum average recorded from *Morus nigra*115. (Table - 8).

The isolated and indentified fungal strains from five barks and they were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium, Penicillium, Alternaria alternate, Trichoderma harianum, Chaetomium globosum, Curvularia lunata, Dactylosporangium macropus, Humicola fuscoatra, Mucor mucedo, Torula herbarum* and *Trichoderma viride* (Table - 9).

In the tropics, only a few studies have been carried out on endophytes of tree species (Frohlich and Hyde, 1999; Rajagopal and Suryanarayanan, 2000; Frohlich and Hyde, 2000) have investigated the endophytic fungi in the leaves of *A. indica*. These studies have shown the effect of leaf tissue type, site and seasonality on endophyte assemblages and colonization. They recorded only *Fusarium* spp. and some sterile fungi. We have recovered endophytic genera like *Curvularia*, *Cochlonema*, *Gliomastix* and *Verticillium* spp., which are reported as endophytes. *Trichoderma*, *Penicillium* and *Pestalotiopsis* spp. were the most dominant endophytes isolated in this study. Endophytic genera such as *Phomopsis*, *Phyllosticta* and *Xylaria* are commonly isolated from tropical and temperate regions (Petrini, 1986). Some species of *Fusarium* are pathogenic to crops, since some phytopathogenic fungus can be modified by mutation to grow as a non-pathogenic endophyte (Freeman and Rodriguez, 1993).

Suresh *et al.,* (1997) were reported the presence of limonoids in the leaf of neem as antifungal and perhaps this is the reason for a low score of endophytes, as reported by (Rajagopal and Suryanarayanan, 2000 ;Frohlich and Hyde, 2000). The occurrence of endophytes seems to be influenced by seasonal variation(Halmschlager *et al.,* 1997). The occurrence of fungal endophytes is mainly influenced by environment and type of host tissue (Rodrigues, 1994). Fungal species like *Trichoderma* are reported to have growth-promoting activity when cultivated with rice seedlings (Mishra *et al.,* 2000). *Penicillium* sps. have been found to produce important antibiotics, which weaken or kill bacteria and other organisms that can cause disease. *Pestalotiopsis* spp. obtained as endophytes in the Himalayan yew (*Taxus wallichiana*) produce taxol, an important chemotherapeutic drug used in the treatment of breast and ovarian cancers (Metz *et al*., 2000). We are currently pursuing fermentation of these microbes to obtain the secondary metabolites to facilitate screening against therapeutic targets as well as against economically important plant pathogens.

SUMMARY

Artocarpus heterophyllus Lam., *Ficus bengalensis* L., *Ficus racemosa* L., *Ficus religiosa* L. and *Morus nigra* L., barks were carried out for this study. The bark containing phytochemical compounds, pharmacognostical behavior and mycoflora were screened in this study. They were : the pharmacognostical behaviour, foreign organic matter was high in *Ficus racemosa*2.26 % and lower 0.7 % in *Artocarpus heterophyllus*. The solubility extractive value percentage was maximum 17.27 % in *Ficus bengalensis* and minimum 12.67 % in *Artocarpus heterophyllus*. Alcohol soluble extractive range between 8.48 and 16.28 % *Ficus racemosa.*The water soluble ash percentage was in between 1.25 - 35% *Ficusreligiosa.*Acid insoluble ash percentage lie between 1.3 *Ficus bengalensis* and 31.63% *Artocarpus heterophyllus.* Total ash content was elevated stage in 18.09% *Morus nigra.* Powder behaviuor with different chemical reagent were mixed with our selected bark powder and after they emitted different colourswere recorded in normal and UV light. *Artocarpus heterophyllus* bark powder contains phytochemicals were screened through such tests,they gave maximum positive results in alkaloid and flavonoids but minimum in proteins and amino acids. *Ficus bengalensis* bark powder contains phytochemicals were screened through such tests, they gave maximum positive results in alkaloid and flavonoids but minimum in proteins and amino acids. *Ficus racemosa* bark powder contains phytochemicals were screened through such tests, they gave maximum negative results in alkaloid and but minimum in flavonoids, proteins, amino acids glycosides. *Ficus religiosa* bark powder contains phytochemicals were screened through such tests, they gave maximum positive results in alkaloid and flavonoids but minimum in proteins and amino acids.

Morus nigra bark powder contains phytochemicals were screened through such tests, they gave maximum negative results in alkaloid and flavonoids but minimum in proteins and amino acids.The bark containing mycorflora were screened they serial dilution factor such an 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} Among the all bark materials *Ficus bengalensis* bark contingmycoflora average was 193.5 and minimum average recorded from *Morus nigra* 115.The isolated and indentified fungal strains were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium sps, Penicillium sps, Alternaria alternate, Trichoderma harianum, Chaetomium globosum, Curvularia lunata, Dactylosporangium macropus, Humicola fuscoatra, Mucor mucedo, Torula herbarum and Trichoderma viride*.

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CONFLICT OF INTEREST	: Nil
Received	: 02.07.2015
Accepted	: 22.10. 2015